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(54) Title: GROWTH DIFFERENTIATION FACTOR-8			
(57) Abstract  A transgenic non-human animal of the species selected from the group consisting of avian, bovine, ovine and porcine having a transgene which results in disrupting the production of and/or activity of growth differentiation factor-8 (GDF-8) chromosomally integrated into the germ cells of the animal is disclosed. Also disclosed are methods for making such animals, and methods of treating animals, including humans, with antibodies or antisense directed to GDF-8. The animals so treated are characterized by increased muscle tissue.			

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**AMENDED CLAIMS**

[received by the International Bureau on 21 July 1998 (21.07.98);  
original claim 2 amended; new claims 49-65 added;  
remaining claims unchanged (4 pages)]

1. A transgenic non-human animal having a transgene disrupting or interfering with expression of growth differentiation factor-8 (GDF-8) chromosomally integrated into the germ cells of the animal.
2. The transgenic animal of claim 1, wherein the animal is selected from the group of species consisting of murine, avian, bovine, ovine, piscine, murine, and porcine.
3. The transgenic animal of claim 1 where the species is avian.
4. The transgenic animal of claim 1 where the species is bovine.
5. The transgenic animal of claim 1 where the species is porcine.
6. The transgenic animal of claim 1 where the species is ovine.
7. The transgenic animal of claim 1 where the species is piscine.
8. The transgenic animal of claim 1, wherein the transgene comprises GDF-8 antisense polynucleotide(s).
9. The transgenic animal of claim 1, wherein the transgene comprises a gene encoding a dominant negative GDF-8 polypeptide.
10. The transgenic animal of claim 1, wherein the animal is homozygous or heterozygous for GDF-8 polynucleotide.
11. A chicken or turkey egg produced by the transgenic animal of claim 3.

44. A method for identifying a compound that affects GDF-8 activity or gene expression comprising:
- a) incubating the compound with GDF-8 polypeptide, or with a recombinant cell expressing GDF-8 under conditions sufficient to allow the components to interact; and
  - b) determining the effect of the compound on GDF-8 activity or expression.
45. The method of claim 44, wherein the effect is inhibition of GDF-8 activity or expression.
46. The method of claim 44, wherein the effect is stimulation of GDF-8 activity or expression.
47. An isolated polynucleotide encoding a truncated GDF-8 polypeptide wherein the truncation is a loss of the C-terminal active fragment of GDF-8.
48. The isolated polynucleotide of claim 47, wherein the polynucleotide is as shown in FIGURE 12a.
49. The transgenic animal of claim 1 where the species is murine.
50. A method for producing a transgenic non-human animal having a phenotype characterized by expression of a transgene otherwise not naturally occurring, wherein expression of the transgene disrupts or interferes with growth differentiation factor-8 (GDF-8) activity, comprising:
- a) introducing a transgene in operable linkage with at least one expression regulatory sequence into a zygote of an animal;
  - b) transplanting the zygote of a) into a pseudopregnant animal;
  - c) allowing the zygote to develop to term; and
  - d) identifying at least one transgenic offspring from c) where expression of the transgene disrupts or interferes with GDF-8 activity.

51. The method of claim 50, wherein the introduction of the transgene into the embryo is by introducing an embryonic stem cell containing the transgene into the embryo.
52. The method of claim 50, wherein the introduction of the transgene into the embryo is by infecting the embryo with a virus containing the transgene.
52. The method of claim 52, wherein the virus is a retrovirus.
53. The method of claim 50, wherein the transgene encodes GDF-8 antisense polynucleotide(s).
54. The method of claim 50, wherein the transgene encodes a dominant negative protein that disrupts or interferes with GDF-8 activity.
55. The method of claim 50, wherein the transgenic animal is homozygous or heterozygous for GDF-8 polynucleotide.
56. The method of claim 50, wherein disrupting or interfering with GDF-8 activity in the transgenic non-human animal produces increased muscle mass as compared to a non-transgenic animal of the same species.
57. The method of claim 50, wherein the animal is selected from the group consisting of murine, avian, bovine, ovine, piscine, and porcine.

58. A method for producing a transgenic non-human animal having a phenotype characterized by expression of a transgene otherwise not naturally occurring, wherein expression of the transgene disrupts or interferes with expression of growth differentiation factor-8 (GDF-8), comprising:
- a) introducing a transgene in operable linkage with at least one expression regulatory sequence into a zygote of an animal;
  - b) transplanting the zygote of a) into a pseudopregnant animal;
  - c) allowing the zygote to develop to term; and
  - d) identifying at least one transgenic offspring from c) where expression of the transgene disrupts or interferes with expression of GDF-8.
59. The method of claim 58, wherein the introduction of the transgene into the embryo is by introducing an embryonic stem cell containing the transgene into the embryo.
60. The method of claim 58, wherein the introduction of the transgene into the embryo is by infecting the embryo with a virus containing the transgene.
61. The method of claim 60, wherein the virus is a retrovirus.
62. The method of claim 58, wherein the transgene encodes GDF-8 antisense polynucleotide(s).
63. The method of claim 58, wherein the transgenic animal is homozygous or heterozygous for GDF-8 polynucleotide.
64. The method of claim 58, wherein disrupting or interfering with GDF-8 activity in the transgenic non-human animal produces increased muscle mass as compared to a non-transgenic animal of the same species.
65. The method of claim 58, wherein the animal is selected from the group consisting of murine, avian, bovine, ovine, piscine, and porcine.



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The invention also provides a method for a method for identifying a compound that affects GDF-8 activity or gene expression including incubating the compound with GDF-8 polypeptide, or with a recombinant cell expressing GDF-8 under conditions sufficient to allow the compounds to interact and determining the effect of the compound on GDF-8 activity or expression.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1a is a Northern blot showing expression of GDF-8 mRNA in adult tissues. The probe was a partial murine GDF-8 clone.

FIGURE 1b is a Southern blot showing GDF-8 genomic sequences identified in mouse, rat, human, monkey, rabbit, cow, pig, dog and chicken.

FIGURE 2 shows partial nucleotide and predicted amino acid sequences of murine GDF-8 (FIGURE 2a; SEQ ID NO:11 and 12, respectively), human GDF-8 (FIGURE 2b; SEQ ID NO: 13 and 14, respectively), rat GDF-8 (FIGURE 2c; SEQ ID NO: 24 and 25, respectively) and chicken GDF-8 (FIGURE 2d; SEQ ID NO: 22 and 23, respectively). The putative dibasic processing sites in the murine sequence are boxed.

FIGURE 3a shows the alignment of the C-terminal sequences of GDF-8 with other members of the TGF- $\beta$  superfamily. The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize alignment.

FIGURE 3b shows the alignment of the C-terminal sequences of GDF-8 from human, murine, rat and chicken sequences.

FIGURE 4 shows amino acid homologies among different members of the TGF superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

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FIGURE 5 shows the sequence of GDF-8. Nucleotide and amino acid sequences of murine (FIGURE 5a and 5b)(GenBank accession number U84005; SEQ ID NO:11 and 12, respectively) and human (FIGURE 5c and 5d; SEQ ID NO:13 and 14, respectively) GDF-8 cDNA clones are shown. Numbers indicate nucleotide position relative to the 5' end. Consensus N-linked glycosylation signals are shaded. The putative RXXR proteolytic cleavage sites are boxed.

FIGURE 6 shows a hydropathicity profile of GDF-8. Average hydrophobicity values for murine (FIGURE 6a) and human (FIGURE 6b) GDF-8 were calculated using the method of Kyte and Doolittle (*J. Mol. Biol.*, 157:105-132, 1982). Positive numbers indicate increasing hydrophobicity.

FIGURE 7 shows a comparison of murine and human GDF-8 amino acid sequences. The predicted murine sequence is shown in the top lines and the predicted human sequence is shown in the bottom lines. Numbers indicate amino acid position relative to the N-terminus. Identities between the two sequences are denoted by a vertical line.

FIGURE 8 shows the expression of GDF-8 in bacteria. BL21 (DE3) (pLysS) cells carrying a pRSET/GDF-8 expression plasmid were induced with isopropylthio- $\beta$ -galactoside, and the GDF-8 fusion protein was purified by metal chelate chromatography. Lanes: total=total cell lysate; soluble=soluble protein fraction; insoluble=insoluble protein fraction (resuspended in 10 Mm Tris pH 8.0, 50 mM sodium phosphate, 8 M urea, and 10 mM  $\beta$ -mercaptoethanol [buffer B]) loaded onto the column, pellet=insoluble protein fraction discarded before loading the column; flow-through=proteins not bound by the column; washes=washes carried out in buffer B at the indicated pH's. Positions of molecular weight standards are shown at the right. Arrow indicates the position of the GDF-8 fusion protein.

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FIGURE 9 shows the expression of GDF-8 in mammalian cells. Chinese hamster ovary cells were transfected with pMSXND/GDF-8 expression plasmids and selected in G418. Conditioned media from G418-resistant cells (prepared from cells transfected with constructs in which GDF-8 was cloned in either the antisense or sense orientation) were concentrated, electrophoresed under reducing conditions, blotted, and probed with anti-GDF-8 antibodies and [<sup>125</sup>I]iodoprotein A. Arrow indicates the position of the processed GDF-8 protein.

FIGURE 10 shows the expression of GDF-8 mRNA. Poly A-selected RNA (5 μg each) prepared from adult tissues (FIGURE 10a) or placentas and embryos (FIGURE 10b) at the indicated days of gestation was electrophoresed on formaldehyde gels, blotted, and probed with full length murine GDF-8.

FIGURE 11 shows chromosomal mapping of human GDF-8. DNA samples prepared from human/rodent somatic cell hybrid lines were subjected to PCR, electrophoresed on agarose gels, blotted, and probed. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the lanes designated M, CHO, and H, the starting DNA template was total genomic DNA from mouse, hamster, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards.

Figure 12a shows a map of the GDF-8 locus (top line) and targeting construct (second line). The black and stippled boxes represent coding sequences for the pro- and C-terminal regions, respectively. The white boxes represent 5' and 3' untranslated sequences. A probe derived from the region downstream of the 3' homology fragment and upstream of the most distal HindIII site shown hybridizes to an 11.2 kb HindIII fragment in the GDF-8 gene and a 10.4 kb fragment in an homologously targeted gene.

Abbreviations: H, HindIII; X, Xba I.

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Figure 12b shows a Southern blot analysis of offspring derived from a mating of heterozygous mutant mice. The lanes are as follows: DNA prepared from wild type 129 SV/J mice (lane 1), targeted embryonic stem cells (lane 2), F1 heterozygous mice (lanes 3 and 4), and offspring derived from a mating of these mice (lanes 5-13).

- 5 Figure 13 shows the muscle fiber size distribution in mutant and wild type littermates. Figure 13a shows the smallest cross-sectional fiber widths measured for wild type (n = 1761) and mutant (n = 1052) tibialis cranial. Figure 13b shows wild type (n = 900) and mutant (n = 900) gastrocnemius muscles, and fiber sizes were plotted as a percent of total fiber number. Standard deviations were 9 and 10  $\mu\text{m}$ , respectively, for wild type and
- 10 mutant tibialis cranial is and 11 and 9  $\mu\text{m}$ , respectively, for wild type and mutant gastrocnemius muscles. Legend: o-o, wild type; \_-\_, mutant.

Figure 14a shows the nucleotide and deduced amino acid sequence for baboon GDF-8 (SEQ ID NO:18 and 19, respectively).

- Figure 14b shows the nucleotide and deduced amino acid sequence for bovine GDF-8
- 15 (SEQ ID NO: 20 and 21, respectively).

Figure 14c shows the nucleotide and deduced amino acid sequence for chicken GDF-8 (SEQ ID NO:22 and 23, respectively).

Figure 14d shows the nucleotide and deduced amino acid sequence for rat GDF-8 (SEQ ID NO:24 and 25, respectively).

- 20 Figure 14e shows the nucleotide and deduced amino acid sequence for turkey GDF-8 (SEQ ID NO:26 and 27, respectively).

Figure 14f shows the nucleotide and deduced amino acid sequence for porcine GDF-8 (SEQ ID NO:28 and 29, respectively).

- Figure 14g shows the nucleotide and deduced amino acid sequence for ovine GDF-8
- 25 (SEQ ID NO:30 and 31, respectively).

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Figures 15a and 15b show an alignment between murine, rat, human, porcine, ovine, baboon, bovine, chicken, and turkey GDF-8 amino acid sequences (SEQ ID NO:12, 25, 14, 29, 31, 19, 21, 23 and 27, respectively).

#### DETAILED DESCRIPTION OF THE INVENTION

- 5 The present invention provides a growth and differentiation factor, GDF-8 and a polynucleotide sequence encoding GDF-8. GDF-8 is expressed at highest levels in muscle and at lower levels in adipose tissue.

The animals contemplated for use in the practice of the subject invention are those animals generally regarded as useful for the processing of food stuffs, *i.e.* avian such as  
10 meat bred and egg laying chicken and turkey, ovine such as lamb, bovine such as beef cattle and milk cows, piscine and porcine. For purposes of the subject invention, these animals are referred to as "transgenic" when such animal has had a heterologous DNA sequence, or one or more additional DNA sequences normally endogenous to the animal (collectively referred to herein as "transgenes") chromosomally integrated into the germ  
15 cells of the animal. The transgenic animal (including its progeny) will also have the transgene integrated into the chromosomes of somatic cells.

The TGF- $\beta$  superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The  
20 structural homology between the GDF-8 protein of this invention and the members of the TGF- $\beta$  family, indicates that GDF-8 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-8 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

- 25 In particular, certain members of this superfamily have expression patterns or possess activities that relate to the function of the nervous system. For example, the inhibins and

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activins have been shown to be expressed in the brain (Meunier, *et al.*, *Proc. Natl. Acad. Sci., USA*, 85:247, 1988; Sawchenko, *et al.*, *Nature*, 334:615, 1988), and activin has been shown to be capable of functioning as a nerve cell survival molecule (Schubert, *et al.*, *Nature*, 344:868, 1990). Another family member, namely, GDF-1, is nervous system-specific in its expression pattern (Lee, S.J., *Proc. Natl. Acad. Sci., USA*, 88:4250, 1991), and certain other family members, such as Vgr-1 (Lyons, *et al.*, *Proc. Natl. Acad. Sci., USA*, 86:4554, 1989; Jones, *et al.*, *Development*, 111:531, 1991), OP-1 (Ozkaynak, *et al.*, *J. Biol. Chem.*, 267:25220, 1992), and BMP-4 (Jones, *et al.*, *Development*, 111:531, 1991), are also known to be expressed in the nervous system. Because it is known that skeletal muscle produces a factor or factors that promote the survival of motor neurons (Brown, *Trends Neurosci.*, 7:10, 1984), the expression of GDF-8 in muscle suggests that one activity of GDF-8 may be as a trophic factor for neurons. In this regard, GDF-8 may have applications in the treatment of neurodegenerative diseases, such as amyotrophic lateral sclerosis or muscular dystrophy, or in maintaining cells or tissues in culture prior to transplantation.

GDF-8 may also have applications in treating disease processes involving muscle, such as in musculodegenerative diseases or in tissue repair due to trauma. In this regard, many other members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been shown to have marked effects on the formation of collagen and to cause a striking angiogenic response in the newborn mouse (Roberts, *et al.*, *Proc. Natl. Acad. Sci., USA* 83:4167, 1986). TGF- $\beta$  has also been shown to inhibit the differentiation of myoblasts in culture (Massague, *et al.*, *Proc. Natl. Acad. Sci., USA* 83:8206, 1986). Moreover, because myoblast cells may be used as a vehicle for delivering genes to muscle for gene therapy, the properties of GDF-8 could be exploited for maintaining cells prior to transplantation or for enhancing the efficiency of the fusion.

The expression of GDF-8 in adipose tissue also raises the possibility of applications for GDF-8 in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. In this regard, TGF- $\beta$  has been shown to be a potent inhibitor of adipocyte

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differentiation *in vitro* (Ignotz and Massague, *Proc. Natl. Acad. Sci., USA* 82:8530, 1985).

**Polypeptides, Polynucleotides, Vectors and Host Cells**

The invention provides substantially pure GDF-8 polypeptide and isolated polynucleo-  
5 tides that encode GDF-8. The term "substantially pure" as used herein refers to GDF-8  
which is substantially free of other proteins, lipids, carbohydrates or other materials with  
which it is naturally associated. One skilled in the art can purify GDF-8 using standard  
techniques for protein purification. The substantially pure polypeptide will yield a single  
major band on a non-reducing polyacrylamide gel. The purity of the GDF-8 polypeptide  
10 can also be determined by amino-terminal amino acid sequence analysis. GDF-8  
polypeptide includes functional fragments of the polypeptide, as long as the activity of  
GDF-8 remains. Smaller peptides containing the biological activity of GDF-8 are  
included in the invention.

The invention provides polynucleotides encoding the GDF-8 protein. These polynucleo-  
15 tides include DNA, cDNA and RNA sequences which encode GDF-8. It is understood  
that all polynucleotides encoding all or a portion of GDF-8 are also included herein, as  
long as they encode a polypeptide with GDF-8 activity. Such polynucleotides include  
naturally occurring, synthetic, and intentionally manipulated polynucleotides. For  
example, GDF-8 polynucleotide may be subjected to site-directed mutagenesis. The  
20 polynucleotide sequence for GDF8 also includes antisense sequences. The polynucleo-  
tides of the invention include sequences that are degenerate as a result of the genetic  
code. There are 20 natural amino acids, most of which are specified by more than one  
codon. Therefore, all degenerate nucleotide sequences are included in the invention as  
long as the amino acid sequence of GDF-8 polypeptide encoded by the nucleotide  
25 sequence is functionally unchanged.

Specifically disclosed herein is a genomic DNA sequence containing a portion of the  
GDF-8 gene. The sequence contains an open reading frame corresponding to the  
predicted C-terminal region of the GDF-8 precursor protein. The encoded polypeptide

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is predicted to contain two potential proteolytic processing sites (KR and RR). Cleavage of the precursor at the downstream site would generate a mature biologically active C-terminal fragment of 109 and 103 amino acids for murine and human species, respectively, with a predicted molecular weight of approximately 12,400. Also disclosed  
5 are full length murine and human GDF-8 cDNA sequences. The murine pre-pro-GDF-8 protein is 376 amino acids in length, which is encoded by a 2676 base pair nucleotide sequence, beginning at nucleotide 104 and extending to a TGA stop codon at nucleotide 1232. The human GDF-8 protein is 375 amino acids and is encoded by a 2743 base pair sequence, with the open reading frame beginning at nucleotide 59 and extending to  
10 nucleotide 1184. GDF-8 is also capable of forming dimers, or heterodimers, with an expected molecular weight of approximately 23-30KD (see Example 4). For example, GDF-8 may form heterodimers with other family members, such as GDF-11.

Also provided herein are the biologically active C-terminal fragments of chicken (Figure 2c) and rat (Figure 2d) GDF-8. The full length nucleotide and deduced amino acid  
15 sequences for baboon, bovine, chicken, rat, ovine, porcine, and turkey are shown in Figures 14a-g and human and murine are shown in Figure 5. As shown in Figure 3b, alignment of the amino acid sequences of human, murine, rat and chicken GDF-8 indicate that the sequences are 100% identical in the C-terminal biologically active fragment. Figure 15 a and 15b also show the alignment of GDF-8 amino acid sequences  
20 for murine, rat, human, baboon, porcine, ovine, bovine, chicken and turkey. Given the extensive conservation of amino acid sequences between species, it would now be routine for one of skill in the art to obtain the GDF-8 nucleic acid and amino acid sequence for GDF-8 from any species, including those provided herein, as well as piscine, for example.

25 The C-terminal region of GDF-8 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily. The GDF-8 sequence contains most of the residues that are highly conserved in other family members and in other species(see FIGURES 3a and 3b and 15 a and 15b). Like the TGF- $\beta$ s and inhibin  $\beta$ s, GDF-8 contains an extra pair of cysteine residues in addition to



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the 7 cysteines found in virtually all other family members. Among the known family members, GDF-8 is most homologous to Vgr-1 (45% sequence identity) (see FIGURE 4).

Minor modifications of the recombinant GDF-8 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-8 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-8 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-8 biological activity.

The nucleotide sequence encoding the GDF-8 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of

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interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-8 polynucleotide of the invention is derived from a mammalian organism, and most preferably from mouse, rat, cow, pig, or human. GDF-8 polynucleo-  
5 tides from chicken, turkey, fish and other species are also included herein. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Given the extensive nucleotide and amino acid homology between species, it would be routine for one of skill in the art to obtain polynucleotides encoding GDF-8 from any species. -  
10 Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the  
15 sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other  
20 words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.* 9:879, 1981).

The development of specific DNA sequences encoding GDF-8 can also be obtained by:  
25 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a doublestranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a

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double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common.

- 5 This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct  
10 synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction  
15 technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA, which have been  
20 denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-8 peptides having at least one epitope, using antibodies specific for GDF-8. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-8 cDNA.

- 25 In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition

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(e.g., GC v. AT content), and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

- 5 An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). Washing can be carried out using only one of these conditions, e.g., high  
10 stringency conditions, or each of the conditions can be used, e.g., for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

- DNA sequences encoding GDF-8 can be expressed *in vitro* by DNA transfer into a  
15 suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously  
20 maintained in the host, are known in the art.

- In the present invention, the GDF-8 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-8 genetic sequences. Such expression vectors contain a  
25 promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited

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to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked  
5 to regulatory elements, for example, a promoter (*e.g.*, T7, metallothionein 1, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-8 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in  
10 prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal region of GDF-8 is expressed from a cDNA clone containing the entire coding sequence of GDF-8. Alternatively, the C-terminal portion of GDF-8 can be  
15 expressed as a fusion protein with the pro- region of another member of the TGF- $\beta$  family or co-expressed with another pro-region (see for example, Hammonds, *et al.*, *Molec. Endocrin.*, 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic,  
20 such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

25 When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-8

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of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic*  
5 *Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

#### 10 GDF-8 Antibodies and Methods of Use

The invention includes antibodies immunoreactive with GDF-8 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the  
15 protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')<sub>2</sub>, Fv and SCA fragments which are capable of binding an epitopic determinant on GDF-8.

(1) An Fab fragment consists of a monovalent antigen-binding fragment of an antibody  
20 molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

(2) An Fab' fragment of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of  
25 an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

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- (3) An (Fab')<sub>2</sub> fragment of an antibody can be obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A (Fab')<sub>2</sub> fragment is a dimer of two Fab' fragments, held together by two disulfide bonds.
- (4) An Fv fragment is defined as a genetically engineered fragment containing the  
5 variable region of a light chain and the variable region of a heavy chain expressed as two chains.
- (5) A single chain antibody ("SCA") is a genetically engineered single chain molecule containing the variable region of a light chain and the variable region of a heavy chain, linked by a suitable, flexible polypeptide linker.
- 10 As used in this invention, the term "epitope" refers to an antigenic determinant on an antigen, such as a GDF-8 polypeptide, to which the paratope of an antibody, such as an GDF-8-specific antibody, binds. Antigenic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three-dimensional structural characteristics, as well as specific charge  
15 characteristics.
- As is mentioned above, antigens that can be used in producing GDF-8-specific antibodies include GDF-8 polypeptides or GDF-8 polypeptide fragments. The polypeptide or peptide used to immunize an animal can be obtained by standard recombinant, chemical synthetic, or purification methods. As is well known in the art, in order to increase  
20 immunogenicity, an antigen can be conjugated to a carrier protein. Commonly used carriers include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit). In addition to such carriers, well known adjuvants can be administered with the antigen to facilitate induction of a strong immune response.
- 25 The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologi-

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cally and genotypically. Malignant cells (*i.e.* cancer) develop as a result of a multistep process. The GDF-8 polynucleotide that is an antisense molecule or that encodes a dominant negative GDF-8 is useful in treating malignancies of the various organ systems, particularly, for example, cells in muscle or adipose tissue. Essentially, any disorder  
5 which is etiologically linked to altered expression of GDF-8 could be considered susceptible to treatment with a GDF-8 agent (*e.g.*, a suppressing or enhancing agent). One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of muscle or adipose tissue which comprises contacting an anti-GDF-8 antibody with a cell suspected  
10 of having a GDF-8 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-8 is labeled with a compound which allows detection of binding to GDF-8. For purposes of the invention, an antibody specific for GDF-8 polypeptide may be used to detect the level of GDF-8 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample  
15 of this invention is muscle tissue. The level of GDF-8 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-8-associated cell proliferative disorder. Such methods of detection are also useful using nucleic acid hybridization to detect the level of GDF-8 mRNA in a sample or to detect an altered GDF-8 gene. Preferably the subject is human.

20 The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of  
25 immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes,



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including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to  
5 detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding  
10 antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent  
15 compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens  
20 as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal  
25 antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

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The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the  
5 best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

10 For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a  
15 particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to i-  
20 mmunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are  $^{111}\text{In}$ ,  $^{97}\text{Ru}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{89}\text{Zr}$  and  $^{201}\text{Tl}$ .

The monoclonal antibodies of the invention can also be labeled with a paramagnetic  
25 isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes

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are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Cr}$ , and  $^{56}\text{Fe}$ .

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-8-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-8-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-8-associated disease in the subject receiving therapy.

#### Additional Methods of Treatment and Diagnosis

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Treatment includes administration of a reagent which modulates activity. The term "modulate" envisions the suppression or expression of GDF-8 when it is over-expressed, or augmentation of GDF-8 expression when it is underexpressed. When a muscle-associated disorder is associated with GDF-8 overexpression, such suppressive reagents as antisense GDF-8 polynucleotide sequence, dominant negative sequences or GDF-8 binding antibody can be introduced into a cell. In addition, an anti-idiotypic antibody which binds to a monoclonal antibody which binds GDF-8 of the invention, or an epitope thereof, may also be used in the therapeutic method of the invention. Alternatively, when a cell proliferative disorder is associated with underexpression or expression of a mutant GDF-8 polypeptide, a sense polynucleotide sequence (the DNA coding strand) or GDF-8 polypeptide can be introduced into the cell. Such muscle-associated disorders include cancer, muscular dystrophy, spinal cord injury, traumatic injury, congestive obstructive pulmonary disease (COPD), AIDS or cachexia. One of skill in the art can determine whether or not a particular therapeutic course of treatment is successful by several methods described herein (e.g., muscle fiber analysis or biopsy). Neurodegenerative

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disorders are also envisioned as treated by the method of the invention. In addition, the method of the invention can be used in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. One of skill in the art can determine whether or not a particular therapeutic course of treatment is successful by several methods described  
5 herein (e.g., muscle fiber analysis or biopsy; determination of fat content). The present examples demonstrate that the methods of the invention are useful for decreasing fat content, and therefore would be useful in the treatment of obesity and related disorders (e.g., diabetes). Neurodegenerative disorders are also envisioned as treated by the method of the invention.

10 Thus, where a cell-proliferative disorder is associated with the expression of GDF-8, nucleic acid sequences that interfere with GDF-8 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-8 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Such disorders include  
15 neurodegenerative diseases, for example. In addition, dominant-negative GDF-8 mutants would be useful to actively interfere with function of "normal" GDF-8.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming  
20 a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded.

Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target GDF-8-producing cell. The use of antisense methods to inhibit the *in vitro*  
25 translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289, 1988).

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Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and  
5 cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. Tetrahymena-type ribozymes recognize  
10 sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences  
15 are preferable to shorter recognition sequences.

In another embodiment of the present invention, a nucleotide sequence encoding a GDF-8 dominant negative protein is provided. For example, a genetic construct that contain such a dominant negative encoding gene may be operably linked to a promoter, such as a tissue-specific promoter. For example, a skeletal muscle specific promoter (*e.g.*,  
20 human skeletal muscle  $\alpha$ -actin promoter) or developmentally specific promoter (*e.g.*, MyHC 3, which is restricted in skeletal muscle to the embryonic period of development, or an inducible promoter (*e.g.*, the orphan nuclear receptor TIS1).

Such constructs are useful in methods of modulating a subject's skeletal mass. For example, a method include transforming an organism, tissue, organ or cell with a genetic  
25 construct encoding a dominant negative GDF-8 protein and suitable promoter in operable linkage and expressing the dominant negative encoding GDF-8 gene, thereby modulating muscle mass by interfering with wild-type GDF-8 activity.

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- GDF-8 most likely forms dimers, homodimers or heterodimers and may even form heterodimers with other GDF family members, such as GDF-11 (*see* Example 4). Hence, while not wanting to be bound by a particular theory, the dominant negative effect described herein may involve the formation of non-functional homodimers or heterodimers of dominant negative and wild-type GDF-8 monomers. More specifically, it is possible that any non-functional homodimer or any heterodimer formed by the dimerization of wild-type and/or dominant negative GDF-8 monomers produces a dominant effect by: 1) being synthesized but not processed or secreted; 2) inhibiting the secretion of wild type GDF-8; 3) preventing normal proteolytic cleavage of the preprotein thereby producing a nonfunctional GDF-8 molecule; 4) altering the affinity of the non-functional dimer (e.g., homodimeric or heterodimeric GDF-8) to a receptor or generating an antagonistic form of GDF-8 that binds a receptor without activating it; or 5) inhibiting the intracellular processing or secretion of GDF-8 related or TGF- $\beta$  family proteins.
- 15 Non-functional GDF-8 can function to inhibit the growth regulating actions of GDF-8 on muscle cells that include a dominant negative GDF-8 gene. Deletion or missense dominant negative forms of GDF-8 that retain the ability to form dimers with wild-type GDF-8 protein but do not function as wild-type GDF-8 proteins may be used to inhibit the biological activity of endogenous wild-type GDF-8. For example, in one embodiment, the proteolytic processing site of GDF-8 may be altered (e.g., deleted) resulting in a GDF-8 molecule able to undergo subsequent dimerization with endogenous wild-type GDF-8 but unable to undergo further processing into a mature GDF-8 form. Alternatively, a non-functional GDF-8 can function as a monomeric species to inhibit the growth regulating actions of GDF-8 on muscle cells.
- 25 Any genetic recombinant method in the art may be used, for example, recombinant viruses may be engineered to express a dominant negative form of GDF-8 which may be used to inhibit the activity of wild-type GDF-8. Such viruses may be used therapeutically for treatment of diseases resulting from aberrant over-expression or activity of GDF-8 protein, such as in denervation hypertrophy or as a means of

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controlling GDF-8 expression when treating disease conditions involving muscle, such as in musculodegenerative diseases or in tissue repair due to trauma or in modulating GDF-8 expression in animal husbandry (e.g., transgenic animals for agricultural purposes).

- 5 The invention provides a method for treating a muscle or adipose tissue disorder in a subject. The method includes administering a therapeutically effective amount of a GDF-8 agent to the subject, thereby inhibiting abnormal growth of muscle or adipose tissue. The GDF-8 agent may include a GDF-8 antisense molecule or a dominant negative polypeptide, for example. A "therapeutically effective amount" of a GDF-8  
10 agent is that amount that ameliorates symptoms of the disorder or inhibits GDF-8 induced growth of muscle, for example, as compared with a normal subject.

#### Gene Therapy

- The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by GDF-8 protein. Such therapy would  
15 achieve its therapeutic effect by introduction of the GDF-8 antisense or dominant negative encoding polynucleotide into cells having the proliferative disorder. Delivery of antisense or dominant negative GDF-8 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense or dominant negative sequences  
20 is the use of targeted liposomes. In contrast, when it is desirable to enhance GDF-8 production, a "sense" GDF-8 polynucleotide or functional equivalent (e.g., the C-term active region) is introduced into the appropriate cell(s).

- Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus.  
25 Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV).

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A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-8 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the GDF-8 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsulation. Helper cell lines which have deletions of the packaging signal include, but are not limited to  $\psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.



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Another targeted delivery system for GDF-8 polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a  
5 liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu\text{m}$  can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active  
10 form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and  
15 substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Manning, *et al.*, *Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly  
20 high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such  
25 as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebroside, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative

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phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-8 in muscle and adipose tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these tissues. Such applications include treatment of cell proliferative disorders involving these and other tissues, such as neural tissue. In addition, GDF-8 may be useful in various gene therapy procedures. In embodiments where GDF-8 polypeptide is administered to a subject, the dosage range is about 0.1 ug/kg to 100 mg/kg; more preferably from about 1 ug/kg to 75 mg/kg and most preferably from about 10 mg/kg to 50 mg/kg.

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### Chromosomal Location of GDF-8

The data in Example 6 shows that the human GDF-8 gene is located on chromosome 2. By comparing the chromosomal location of GDF-8 with the map positions of various human disorders, it should be possible to determine whether mutations in the GDF-8 gene are involved in the etiology of human diseases. For example, an autosomal recessive form of juvenile amyotrophic lateral sclerosis has been shown to map to chromosome 2 (Hentati, *et al.*, *Neurology*, 42 [Suppl.3]:201, 1992). More precise mapping of GDF-8 and analysis of DNA from these patients may indicate that GDF-8 is, in fact, the gene affected in this disease. In addition, GDF-8 is useful for distinguishing chromosome 2 from other chromosomes.

### Transgenic Animals and Methods of Making the same

Various methods to make the transgenic animals of the subject invention can be employed. Generally speaking, three such methods may be employed. In one such method, an embryo at the pronuclear stage (a "one cell embryo") is harvested from a female and the transgene is microinjected into the embryo, in which case the transgene will be chromosomally integrated into both the germ cells and somatic cells of the resulting mature animal. In another such method, embryonic stem cells are isolated and the transgene incorporated therein by electroporation, plasmid transfection or microinjection, followed by reintroduction of the stem cells into the embryo where they colonize and contribute to the germ line. Methods for microinjection of mammalian species is described in United States Patent No. 4,873,191. In yet another such method, embryonic cells are infected with a retrovirus containing the transgene whereby the germ cells of the embryo have the transgene chromosomally integrated therein. When the animals to be made transgenic are avian, because avian fertilized ova generally go through cell division for the first twenty hours in the oviduct, microinjection into the pronucleus of the fertilized egg is problematic due to the inaccessibility of the pronucleus. Therefore, of the methods to make transgenic animals described generally above, retrovirus infection is preferred for avian species, for example as described in U.S. 5,162,215. If microinjection is to be used with avian species, however, a recently published procedure by Love *et al.*, (*Biotechnology*, 12, Jan 1994) can be utilized

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whereby the embryo is obtained from a sacrificed hen approximately two and one-half hours after the laying of the previous laid egg, the transgene is microinjected into the cytoplasm of the germinal disc and the embryo is cultured in a host shell until maturity. When the animals to be made transgenic are bovine or porcine, microinjection can be  
5 hampered by the opacity of the ova thereby making the nuclei difficult to identify by traditional differential interference-contrast microscopy. To overcome this problem, the ova can first be centrifuged to segregate the pronuclei for better visualization.

The "non-human animals" of the invention bovine, porcine, ovine and avian animals (e.g., cow, pig, sheep, chicken, turkey). The "transgenic non-human animals" of the  
10 invention are produced by introducing "transgenes" into the germline of the non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The zygote is the best target for micro-injection. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the  
15 injected DNA will be incorporated into the host gene before the first cleavage (Brinster *et al.*, *Proc. Natl. Acad. Sci. USA* 82:4438-4442, 1985). As a consequence, all cells of the transgenic non-human animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene.

20 The term "transgenic" is used to describe an animal which includes exogenous genetic material within all of its cells. A "transgenic" animal can be produced by cross-breeding two chimeric animals which include exogenous genetic material within cells used in reproduction. Twenty-five percent of the resulting offspring will be transgenic *i.e.*, animals which include the exogenous genetic material within all of their cells in both  
25 alleles. 50% of the resulting animals will include the exogenous genetic material within one allele and 25% will include no exogenous genetic material.

In the microinjection method useful in the practice of the subject invention, the transgene is digested and purified free from any vector DNA *e.g.* by gel electrophoresis. It is

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preferred that the transgene include an operatively associated promoter which interacts with cellular proteins involved in transcription, ultimately resulting in constitutive expression. Promoters useful in this regard include those from cytomegalovirus (CMV), Moloney leukemia virus (MLV), and herpes virus, as well as those from the genes  
5 encoding metallothionin, skeletal actin, P-enolpyruvate carboxylase (PEPCK), phosphoglycerate (PGK), DHFR, and thymidine kinase. Promoters for viral long terminal repeats (LTRs) such as Rous Sarcoma Virus can also be employed. When the animals to be made transgenic are avian, preferred promoters include those for the chicken  $\beta$ -globin gene, chicken lysozyme gene, and avian leukosis virus. Constructs  
10 useful in plasmid transfection of embryonic stem cells will employ additional regulatory elements well known in the art such as enhancer elements to stimulate transcription, splice acceptors, termination and polyadenylation signals, and ribosome binding sites to permit translation.

Retroviral infection can also be used to introduce transgene into a non-human animal, as  
15 described above. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retro viral infection (Jaenich, R., Proc. Natl. Acad. Sci USA 73:1260-1264, 1976). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan, *et al.* (1986) in *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press,  
20 Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retro virus carrying the transgene (Jahner, *et al.*, Proc. Natl. Acad. Sci. USA 82:6927-6931, 1985; Van der Putten, *et al.*, Proc. Natl. Acad. Sci USA 82:6148-6152, 1985). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, *supra*; Stewart,  
25 *et al.*, EMBO J. 6:383-388, 1987). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (D. Jahner *et al.*, Nature 298:623-628, 1982). Most of the founders will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic nonhuman animal. Further, the founder may contain various retro viral insertions of the transgene  
30 at different positions in the genome which generally will segregate in the offspring. In

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addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (D. Jahner *et al.*, *supra*).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES  
5 cells are obtained from pre-implantation embryos cultured *in vitro* and fused with  
embryos (M. J. Evans *et al.* *Nature* 292:154-156, 1981; M.O. Bradley *et al.*, *Nature* 309:  
255-258, 1984; Gossler, *et al.*, *Proc. Natl. Acad. Sci USA* 83: 9065-9069, 1986; and  
Robertson *et al.*, *Nature* 322:445-448, 1986). Transgenes can be efficiently introduced  
into the ES cells by DNA transfection or by retro virus-mediated transduction. Such  
10 transformed ES cells can thereafter be combined with blastocysts from a nonhuman  
animal. The ES cells thereafter colonize the embryo and contribute to the germ line of  
the resulting chimeric animal. (For review see Jaenisch, R., *Science* 240: 1468-1474,  
1988).

"Transformed" means a cell into which (or into an ancestor of which) has been  
15 introduced, by means of recombinant nucleic acid techniques, a heterologous nucleic acid  
molecule. "Heterologous" refers to a nucleic acid sequence that either originates from  
another species or is modified from either its original form or the form primarily  
expressed in the cell.

"Transgene" means any piece of DNA which is inserted by artifice into a cell, and  
20 becomes part of the genome of the organism (*i.e.*, either stably integrated or as a stable  
extrachromosomal element) which develops from that cell. Such a transgene may  
include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic  
organism, or may represent a gene homologous to an endogenous gene of the organism.  
Included within this definition is a transgene created by the providing of an RNA  
25 sequence which is transcribed into DNA and then incorporated into the genome. The  
transgenes of the invention include DNA sequences which encode GDF-8, and include  
GDF-sense, antisense, dominant negative encoding polynucleotides, which may be  
expressed in a transgenic non-human animal. The term "transgenic" as used herein

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additionally includes any organism whose genome has been altered by *in vitro* manipulation of the early embryo or fertilized egg or by any transgenic technology to induce a specific gene knockout. The term "gene knockout" as used herein, refers to the targeted disruption of a gene *in vivo* with complete loss of function that has been

5 achieved by any transgenic technology familiar to those in the art. In one embodiment, transgenic animals having gene knockouts are those in which the target gene has been rendered nonfunctional by an insertion targeted to the gene to be rendered non-functional by homologous recombination. As used herein, the term "transgenic" includes any transgenic technology familiar to those in the art which can produce an organism

10 carrying an introduced transgene or one in which an endogenous gene has been rendered non-functional or "knocked out." An example of a transgene used to "knockout" GDF-8 function in the present Examples is described in Example 8 and FIGURE 12a. Thus, in another embodiment, the invention provides a transgene wherein the entire mature C-terminal region of GDF-8 is deleted.

15 The transgene to be used in the practice of the subject invention is a DNA sequence comprising a modified GDF-8 coding sequence. In a preferred embodiment, the GDF-8 gene is disrupted by homologous targeting in embryonic stem cells. For example, the entire mature C-terminal region of the GDF-8 gene may be deleted as described in the examples below. Optionally, the GDF-8 disruption or deletion may be accompanied by

20 insertion of or replacement with other DNA sequences, such as a non-functional GDF-8 sequence. In other embodiments, the transgene comprises DNA antisense to the coding sequence for GDF-8. In another embodiment, the transgene comprises DNA encoding an antibody or receptor peptide sequence which is able to bind to GDF-8. The DNA and peptide sequences of GDF-8 are known in the art, the sequences, localization and activity

25 disclosed in WO94/21681 and pending United States patent application 08/033,923, filed on March 19, 1993, incorporated by reference in its entirety. The disclosure of both of these applications are hereby incorporated herein by reference. Where appropriate, DNA sequences that encode proteins having GDF-8 activity but differ in nucleic acid sequence due to the degeneracy of the genetic code may also be used herein, as may truncated

30 forms, allelic variants and interspecies homologues.

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The invention also includes animals having heterozygous mutations in GDF-8 or partial inhibition of GDF-8 function or expression. A heterozygote would exhibit an intermediate increase in muscle mass as compared to the homozygote as shown in Table 4 below. In other words, partial loss of function leads to a partial increase in muscle mass. One of skill in the art would readily be able to determine if a particular mutation or if an antisense molecule was able to partially inhibit GDF-8. For example, *in vitro* testing may be desirable initially by comparison with wild-type or untreated GDF-8 (e.g., comparison of northern blots to examine a decrease in expression).

After an embryo has been microinjected, colonized with transfected embryonic stem cells or infected with a retrovirus containing the transgene (except for practice of the subject invention in avian species which is addressed elsewhere herein) the embryo is implanted into the oviduct of a pseudopregnant female. The consequent progeny are tested for incorporation of the transgene by Southern blot analysis of blood samples using transgene specific probes. PCR is particularly useful in this regard. Positive progeny (G0) are crossbred to produce offspring (G1) which are analyzed for transgene expression by Northern blot analysis of tissue samples. To be able to distinguish expression of like-species transgenes from expression of the animals endogenous GDF-8 gene(s), a marker gene fragment can be included in the construct in the 3' untranslated region of the transgene and the Northern probe designed to probe for the marker gene fragment. The serum levels of GDF-8 can also be measured in the transgenic animal to establish appropriate expression. Expression of the GDF-8 transgenes, thereby decreasing the GDF-8 in the tissue and serum levels of the transgenic animals and consequently increasing the muscle tissue content results in the foodstuffs from these animals (*i.e.* eggs, beef, pork, poultry meat, milk, *etc.*) having markedly increased muscle content, and preferably without increased, and more preferably, reduced levels of fat and cholesterol. By practice of the subject invention, a statistically significant increase in muscle content, preferably at least a 2% increase in muscle content (e.g., in chickens), more preferably a 25% increase in muscle content as a percentage of body weight, more preferably greater than 40% increase in muscle content in these foodstuffs can be obtained.



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Additional Methods of Use

Thus, the present invention includes methods for increasing muscle mass in domesticated animals, characterized by inactivation or deletion of the gene encoding growth and differentiation factor-8 (GDF-8). The domesticated animal is preferably selected from the group consisting of ovine, bovine, porcine, piscine and avian. The animal may be treated with an isolated polynucleotide sequence encoding growth and differentiation factor-8 which polynucleotide sequence is also from a domesticated animal selected from the group consisting of ovine, bovine, porcine, piscine and avian. The present invention includes methods for increasing the muscle mass in domesticated animals characterized by administering to a domesticated animal monoclonal antibodies directed to the GDF-8 polypeptide. The antibody may be an anti-GDF-8, and may be either a monoclonal antibody or a polyclonal antibody.

The invention includes methods comprising using an anti-GDF-8 monoclonal antibody, antisense, or dominant negative mutants as a therapeutic agent to inhibit the growth regulating actions of GDF-8 on muscle cells. Muscle cells are defined to include fetal or adult muscle cells, as well as progenitor cells which are capable of differentiation into muscle. The monoclonal antibody may be a humanized (*e.g.*, either fully or a chimeric) monoclonal antibody, of any species origin, such as murine, ovine, bovine, porcine or avian. Methods of producing antibody molecules with various combinations of "humanized" antibodies are well known in the art and include combining murine variable regions with human constant regions (Cabily, *et al. Proc.Natl.Acad.Sci. USA*, 81:3273, 1984), or by grafting the murine-antibody complementary determining regions (CDRs) onto the human framework (Richmann, *et al., Nature* 332:323, 1988). Other general references which teach methods for creating humanized antibodies include Morrison, *et al., Science*, 229:1202, 1985; Jones, *et al., Nature*, 321:522, 1986; Monroe, *et al., Nature* 312:779, 1985; Oi, *et al., BioTechniques*, 4:214, 1986; European Patent Application No. 302,620; and U.S. Patent No. 5,024,834. Therefore, by humanizing the monoclonal antibodies of the invention for *in vivo* use, an immune response to the antibodies would be greatly reduced.

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The monoclonal antibody, GDF-8 polypeptide, or GDF-8 polynucleotide (all "GDF-8 agents") may have the effect of increasing the development of skeletal muscles. In preferred embodiments of the claimed methods, the GDF-8 monoclonal antibody, polypeptide, or polynucleotide is administered to a patient suffering from a disorder selected from the group consisting of muscle wasting disease, neuromuscular disorder, muscle atrophy or aging. The GDF-8 agent may also be administered to a patient suffering from a disorder selected from the group consisting of muscular dystrophy, spinal cord injury, traumatic injury, congestive obstructive pulmonary disease (COPD), AIDS or cachexia. In a preferred embodiment, the GDF-8 agent is administered to a patient with muscle wasting disease or disorder by intravenous, intramuscular or subcutaneous injection; preferably, a monoclonal antibody is administered within a dose range between about 0.1 mg/kg to about 100 mg/kg; more preferably between about 1 ug/kg to 75 mg/kg; most preferably from about 10 mg/kg to 50 mg/kg. The antibody may be administered, for example, by bolus injection or by slow infusion. Slow infusion over a period of 30 minutes to 2 hours is preferred. The GDF-8 agent may be formulated in a formulation suitable for administration to a patient. Such formulations are known in the art.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the GDF-8 protein, *e.g.* amount of tissue desired to be formed, the site of tissue damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the types of agent, such as anti-GDF-8 antibodies, to be used in the composition. Generally, systemic or injectable administration, such as intravenous (IV), intramuscular (IM) or subcutaneous (Sub-Q) injection. Administration will generally be initiated at a dose which is minimally effective, and the dose will be increased over a preselected time course until a positive effect is observed. Subsequently, incremental increases in dosage will be made limiting such incremental increases to such levels that produce a corresponding increase in effect, while taking into account any adverse affects that may appear. The addition of other known growth

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factors, such as IGF I (insulin like growth factor I), human, bovine, or chicken growth hormone which may aid in increasing muscle mass, to the final composition, may also affect the dosage. In the embodiment where an anti-GDF-8 antibody is administered, the anti-GDF-8 antibody is generally administered within a dose range of about 0.1 ug/kg  
5 to about 100 mg/kg.; more preferably between about 10 mg/kg to 50 mg/kg.

Progress can be monitored by periodic assessment of tissue growth and/or repair. The progress can be monitored, for example, x-rays, histomorphometric determinations and tetracycline labeling.

#### Screening for GDF-8 Modulating Compounds

- 10 In another embodiment, the invention provides a method for identifying a compound or molecule that modulates GDF-8 protein activity or gene expression. The method includes incubating components comprising the compound, GDF-8 polypeptide or with a recombinant cell expressing GDF-8 polypeptide, under conditions sufficient to allow the components to interact and determining the effect of the compound on GDF-8  
15 activity or expression. The effect of the compound on GDF-8 activity can be measured by a number of assays, and may include measurements before and after incubating in the presence of the compound. Compounds that affect GDF-8 activity or gene expression include peptides, peptidomimetics, polypeptides, chemical compounds and biologic agents. Assays include Northern blot analysis of GDF-8 mRNA (for gene expression),  
20 Western blot analysis (for protein level) and muscle fiber analysis (for protein activity).

The above screening assays may be used for detecting the compounds or molecules that bind to the GDF-8 receptor or GDF-8 polypeptide, in isolating molecules that bind to the GDF-8 gene, for measuring the amount of GDF-8 in a sample, either polypeptide or RNA (mRNA), for identifying molecules that may act as agonists or antagonists, and the like.  
25 For example, GDF-8 antagonists are useful for treatment of muscular and adipose tissue disorders (e.g., obesity).

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Incubating includes conditions which allow contact between the test compound and GDF-8 polypeptide or with a recombinant cell expressing GDF-8 polypeptide. Contacting includes in solution and in solid phase, or in a cell. The test compound may optionally be a combinatorial library for screening a plurality of compounds. Compounds identified in the method of the invention can be further evaluated, detected, 5 cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a specific DNA sequence such as PCR, oligomer restriction (Saiki, *et al.*, *Bio/Technology*, 3:1008-1012, 1985), allele-specific oligonucleotide (ASO) probe analysis (Conner, *et al.*, *Proc. Natl. Acad. Sci. USA*, 10 80:278, 1983), oligonucleotide Landegren, *et al.*, *Science*, 241:1077, 1988), and the like. Molecular techniques for DNA analysis have been reviewed (Landegren, *et al.*, *Science*, 242:229-237, 1988).

All references cited herein are hereby incorporated by reference in their entirety.

The following examples are intended to illustrate but not limit the invention. While they 15 are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

#### **EXAMPLE 1**

#### **IDENTIFICATION AND ISOLATION OF A NOVEL**

#### **TGF- $\beta$ FAMILY MEMBER**

20 To identify a new member of the TGF- $\beta$  superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family members: one region spanning the two tryptophan residues conserved in all family members except MIS and the other region spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on mouse 25 genomic DNA followed by subcloning the PCR products using restriction sites placed at the 5' ends of the primers, picking individual *E. coli* colonies carrying these subcloned

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inserts, and using a combination of random sequencing and hybridization analysis to eliminate known members of the superfamily.

GDF-8 was identified from a mixture of PCR products obtained with the primers

SJL141: 5'-CCGGAATTCGGITGG(G/C/A)A(G/A/T/C)(A/G)A(T/C)TGG(A/G)TI

5 (A/G)TI(T/G)CICC-3' (SEQ ID NO:1)

SJL147:

5'-CCGGAATTC(G/A)CAI(G/C)C(G/A)CA(G/A)CT(G/A/T/C)

TCIACI(G/A)(T/C)CAT-3' (SEQ ID NO:2)

10 PCR using these primers was carried out with 2 µg mouse genomic DNA at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min for 40 cycles.

15 PCR products of approximately 280 bp were gel-purified, digested with Eco RI, gel-purified again, and subcloned in the Bluescript vector (Stratagene, San Diego, CA). Bacterial colonies carrying individual subclones were picked into 96 well microtiter plates, and multiple replicas were prepared by plating the cells onto nitrocellulose. The replicate filters were hybridized to probes representing known members of the family, and DNA was prepared from nonhybridizing colonies for sequence analysis.

20 The primer combination of SJL141 and SJL147, encoding the amino acid sequences GW(H/Q/N/K/D/E)(D/N)W(V/I/M)(V/I/M)(A/S)P (SEQ ID NO:9) and M(V/I/M/T/A)V(D/E)SC(G/A)C (SEQ ID NO:10), respectively, yielded four previously identified sequences (BMP-4, inhibin,βB, GDF-3 and GDF-5) and one novel sequence, which was designated GDF-8, among 110 subclones analyzed.

Human GDF-8 was isolated using the primers:

ACM13: 5'-CGCGGATCCAGAGTCAAGGTGACAGACACAC-3' (SEQ ID NO:3); and

ACM14: 5'-CGCGGATCCTCCTCATGAGCACCCACAGCGGTC-3' (SEQ ID NO:4)

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PCR using these primers was carried out with one  $\mu$ g human genomic DNA at 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min for 30 cycles. The PCR product was digested with Bam HI, gel-purified, and subcloned in the Bluescript vector (Stratagene, San Francisco, CA).

5

## EXAMPLE 2

### EXPRESSION PATTERN AND SEQUENCE OF GDF-8

To determine the expression pattern of GDF-8, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. RNA isolation and Northern analysis were carried out as described previously (Lee, S.J., *Mol. Endocrinol.*, 4:1034, 1990) except that hybridization was carried out in 5X SSPE, 10% dextran sulfate, 50% formamide, 1% SDS, 200  $\mu$ g/ml salmon DNA, and 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. Five micrograms of twice poly A-selected RNA prepared from each tissue (except for muscle, for which only 2  $\mu$ g RNA was used) were electrophoresed on formaldehyde gels, blotted, and probed with GDF-8. As shown in FIGURE 1, the GDF-8 probe detected a single mRNA species expressed at highest levels in muscle and at significantly lower levels in adipose tissue.

To obtain a larger segment of the GDF-8 gene, a mouse genomic library was screened with a probe derived from the GDF-8 PCR product. The partial sequence of a GDF-8 genomic clone is shown in FIGURE 2a. The sequence contains an open reading frame corresponding to the predicted C-terminal region of the GDF-8 precursor protein. The predicted GDF-8 sequence contains two potential proteolytic processing sites, which are boxed. Cleavage of the precursor at the second of these sites would generate a mature C terminal fragment 109 amino acids in length with a predicted molecular weight of 12,400. The partial sequence of human GDF-8 is shown in FIGURE 2b. Assuming no PCR-induced errors during the isolation of the human clone, the human and mouse amino acid sequences in this region are 100% identical.

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The C-terminal region of GDF-8 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily (FIGURE 3). FIGURE 3 shows the alignment of the C-terminal sequences of GDF-8 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 5 1991), human BMP-2 and 4 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human Vgr-1 (Celeste, *et al.* *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, *et al.*, *EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human BMP-3 (Wozney, *et al.*, *Science*, 242:1528-1534, 1988), human MiS (Cate, *et al.* *Cell*, 45:685-698, 1986), human inhibin 10 alpha,  $\beta$ A, and  $\beta$ B (Mason, *et al.*, *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), human TGF- $\beta$ 1 (Derynck, *et al.*, *Nature*, 316:701-705, 1985), human TGF- $\beta$ 2 (deMartin, *et al.*, *EMBO J.*, 6:3673-3677, 1987), and human TGF- $\beta$ 3 (ten Dijke, *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

15 GDF-8 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing. Like the TGF- $\beta$ s and inhibin  $\beta$ s, GDF-8 also contains two additional cysteine residues. In the case of TGF- $\beta$ 2, these two additional cysteine residues are known to form an intramolecular disulfide bond (Daopin, *et al.*, *Science*, 257:369, 1992; Schlunegger and Grutter, *Nature*, 20 358:430, 1992).

FIGURE 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C terminus. Boxes represent homologies among highly-related members within particular subgroups. In this region, 25 GDF-8 is most homologous to Vgr-1 (45% sequence identity).

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**EXAMPLE 3****ISOLATION OF cDNA CLONES ENCODING MURINE AND HUMAN GDF-8**

In order to isolate full-length cDNA clones encoding murine and human GDF-8, cDNA libraries were prepared in the lambda ZAP II vector (Stratagene) using RNA prepared from skeletal muscle. From 5 µg of twice poly A-selected RNA prepared from murine and human muscle, cDNA libraries consisting of 4.4 million and 1.9 million recombinant phage, respectively, were constructed according to the instructions provided by Stratagene. These libraries were screened without amplification. Library screening and characterization of cDNA inserts were carried out as described previously (Lee, *Mol. Endocrinol.*, 4:1034-1040).

From  $2.4 \times 10^6$  recombinant phage screened from the murine muscle cDNA library, greater than 280 positive phage were identified using a murine GDF-8 probe derived from a genomic clone, as described in Example 1. The entire nucleotide sequence of the longest cDNA insert analyzed is shown in FIGURE 5a and 5b and SEQ ID NO:11. The 2676 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotide 104 and extending to a TGA stop codon at nucleotide 1232. Upstream of the putative initiating methionine codon is an in-frame stop codon at nucleotide 23. The predicted pre-pro-GDF-8 protein is 76 amino acids in length. The sequence contains a core of hydrophobic amino acids at the N-terminus suggestive of a signal peptide for secretion (FIGURE 6a), one potential N-glycosylation site at asparagine 72, a putative RXXR proteolytic cleavage site at amino acids 264-267, and a C-terminal region showing significant homology to the known members of the TGF- $\beta$  superfamily. Cleavage of the precursor protein at the putative RXXR site would generate a mature C-terminal GDF-8 fragment 109 amino acids in length with a predicted molecular weight of approximately 12,400.

From  $1.9 \times 10^6$  recombinant phage screened from the human muscle cDNA library, 4 positive phage were identified using a human GDF-8 probe derived by polymerase chain reaction on human genomic DNA. The entire nucleotide sequence of the longest cDNA



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insert is shown in FIGURE 5c and 5d and SEQ ID NO:13. The 2743 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotide 59 and extending to a TGA stop codon at nucleotide 1184. The predicted pre-pro-GDF-8 protein is 375 amino acids in length. The sequence contains a core of  
5 hydrophobic amino acids at the N-terminus suggestive of a signal peptide for secretion (FIGURE 6b), one potential N-glycosylation site at asparagine 71, and a putative RX(R proteolytic cleavage site at amino acids 263-266. FIGURE 7 shows a comparison of the predicted murine (top) and human (bottom) GDF-8 amino acid sequences. Numbers indicate amino acid position relative to the N-terminus. Identities between the two  
10 sequences are denoted by a vertical line. Murine and human GDF-8 are approximately 94% identical in the predicted pro-regions and 100% identical following the predicted RXXR cleavage sites.

#### EXAMPLE 4

##### DIMERIZATION OF GDF-8

15 To determine whether the processing signals in the GDF-8 sequence are functional and whether GDF-8 forms dimers like other members of the TGF- $\beta$  superfamily, the GDF-8 cDNA was stably expressed in CHO cells. The GDF-8 coding sequence was cloned into the pMSXND expression vector (Lee and Nathans, *J. Biol. Chem.*, 263:3521, (1988) and transfected into CHO cells. Following G418 selection, the cells were selected in 0.2  $\mu$ M  
20 methotrexate, and conditioned medium from resistant cells was concentrated and electrophoresed on SDS gels. Conditioned medium was prepared by Cell Trends, Inc. (Middletown, MD). For preparation of anti-GDF-8 serum, the C-terminal region of GDF-8 (amino acids 268 to 376) was expressed in bacteria using the RSET vector (Invitrogen, San Diego, CA), purified using a nickle chelate column, and injected into  
25 rabbits. All immunizations were carried out by Spring Valley Labs (Woodbine, MD). Western analysis using [ $^{125}$ I]iodoprotein A was carried out as described (Burnette, W.N., *Anal. Biochem.*, 112:195, 1981). Western analysis of conditioned medium prepared from these cells using an antiserum raised against a bacterially-expressed C-terminal fragment of GDF-8 detected two protein species with apparent molecular weights of approximately

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52K and 15K under reducing conditions, consistent with unprocessed and processed forms of GDF-8, respectively. No bands were obtained either with preimmune serum or with conditioned medium from CHO cells transfected with an antisense construct. Under non-reducing conditions, the GDF-8 antiserum detected two predominant protein species with apparent molecular weights of approximately 101K and 25K, consistent with dimeric forms of unprocessed and processed GDF-8, respectively. Hence, like other TGF- $\beta$  family members, GDF-8 appears to be secreted and proteolytically processed, and the C-terminal region appears to be capable of forming a disulfide-linked dimer.

#### EXAMPLE 5

#### PREPARATION OF ANTIBODIES AGAINST GDF-8 AND EXPRESSION OF GDF-8 IN MAMMALIAN CELLS

In order to prepare antibodies against GDF-8, GDF-8 antigen was expressed as a fusion protein in bacteria. A portion of murine GDF-8 cDNA spanning amino acids 268-376 (mature region) was inserted into the pRSET vector (Invitrogen) such that the GDF-8 coding sequence was placed in frame with the initiating methionine codon present in the vector; the resulting construct created an open reading frame encoding a fusion protein with a molecular weight of approximately 16,600. The fusion construct was transformed into BL21 (DE3) (pLysS) cells, and expression of the fusion protein was induced by treatment with isopropylthio- $\beta$ -galactoside as described (Rosenberg, *et al.*; *Gene*, 56:125-135). The fusion protein was then purified by metal chelate chromatography according to the instructions provided by Invitrogen. A Coomassie blue-stained gel of unpurified and purified fusion proteins is shown in FIGURE 8.

The purified fusion protein was used to immunize both rabbits and chickens. Immunization of rabbits was carried out by Spring Valley Labs (Sykesville, MD), and immunization of chickens was carried out by HRP, Inc. (Denver, PA). Western analysis of sera both from immunized rabbits and from immunized chickens demonstrated the presence of antibodies directed against the fusion protein.

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To express GDF-8 in mammalian cells, the murine GDF-8 cDNA sequence from nucleotides 48-1303 was cloned in both orientations downstream of the metallothionein I promoter in the pMSXND expression vector; this vector contains processing signals derived from SV40, a dihydrofolate reductase gene, and a gene conferring resistance to the antibiotic G418 (Lee and Nathans, *J. Biol. Chem.*, 263:3521-3527). The resulting constructs were transfected into Chinese hamster ovary cells, and stable transfectants were selected in the presence of G418. Two milliliters of conditioned media prepared from the G418-resistant cells were dialyzed, lyophilized, electrophoresed under denaturing, reducing conditions, transferred to nitrocellulose, and incubated with anti-GDF-8 antibodies (described above) and [<sup>125</sup>I]iodoproteinA.

As shown in FIGURE 9, the rabbit GDF-8 antibodies (at a 1:500 dilution) detected a protein of approximately the predicted molecular weight for the mature C-terminal fragment of GDF-8 in the conditioned media of cells transfected with a construct in which GDF-8 had been cloned in the correct (sense) orientation with respect to the metallothionein promoter (lane 2); this band was not detected in a similar sample prepared from cells transfected with a control antisense construct (lane 1). Similar results were obtained using antibodies prepared in chickens. Hence, GDF-8 is secreted and proteolytically processed by these transfected mammalian cells.

#### EXAMPLE 6

#### EXPRESSION PATTERN OF GDF-8

To determine the pattern of GDF-8, 5 µg of twice poly A-selected RNA prepared from a variety of murine tissue sources were subjected to Northern analysis. As shown in FIGURE 10a (and as shown previously in Example 2), the GDF-8 probe detected a single mRNA species present almost exclusively in skeletal muscle among a large number of adult tissues surveyed. On longer exposures of the same blot, significantly lower but detectable levels of GDF-8 mRNA were seen in fat, brain, thymus, heart, and lung. Hence, these results confirm the high degree of specificity of GDF-8 expression in skeletal muscle. GDF-8 mRNA was also detected in mouse embryos at both gestational

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ages (day 12.5 and day 18.5 post-coital) examined but not in placentas at various stages of development (FIGURE 10b).

To further analyze the expression pattern of GDF-8, *in situ* hybridization was performed on mouse embryos isolated at various stages of development.

- 5 For all *in situ* hybridization experiments, probes corresponding to the C-terminal region of GDF-8 were excluded in order to avoid possible cross-reactivity with other members of the superfamily. Whole mount *in situ* hybridization analysis was carried out as described (Wilkinson, D.G., *In Situ Hybridization, A Practical Approach*, pp. 75-83, IRL Press, Oxford, 1992) except that blocking and antibody incubation steps were
- 10 carried out as in Knecht *et al.* (Knecht, *et al.*, *Development*, 121:1927, 1995). Alkaline phosphatase reactions were carried out for 3 hours for day 10.5 embryos and overnight for day 9.5 embryos. Hybridization was carried out using digoxigenin- labelled probes spanning nucleotides 8-811 and 1298-2676, which correspond to the pro-region and 3' untranslated regions, respectively. *In situ* hybridization to sections was carried out as
- 15 described (Wilkinson, *et al.*, *Cell*, 50:79, 1987) using <sup>35</sup>S-labelled probes ranging from approximately 100-650 bases in length and spanning nucleotides 8-793 and 1566-2595. Following hybridization and washing, slides were dipped in NTB-3 photographic emulsion, exposed for 16-19 days, developed and stained with either hematoxylin and eosin or toluidine blue. RNA isolation, poly A selection, and Northern analysis were
- 20 carried out as described previously (McPherron and Lee, *J. Biol. Chem.*, 268:3444, 1993).

At all stages examined, the expression of GDF-8 mRNA appeared to be restricted to developing skeletal muscle. At early stages, GDF-8 expression was restricted to developing somites. By whole mount *in situ* hybridization analysis, GDF-8 mRNA could

25 first be detected as early as day 9.5 post coitum in approximately one-third of the somites. At this stage of development, hybridization appeared to be restricted to the most mature (9 out of 21 in this example), rostral somites. By day 10.5 p.c., GDF-8 expression was clearly evident in almost every somite (28 out of 33 in this example

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shown). Based on *in situ* hybridization analysis of sections prepared from day 10.5 p.c. embryos, the expression of GDF-8 in somites appeared to be localized to the myotome compartment. At later stages of development, GDF-8 expression was detected in a wide range of developing muscles.

- 5 GDF-8 continues to be expressed in adult animals as well. By Northern analysis, GDF-8 mRNA expression was seen almost exclusively in skeletal muscle among the different adult tissues examined. A significantly lower though clearly detectable signal was also seen in adipose tissue. Based on Northern analysis of RNA prepared from a large number of different adult skeletal muscles, GDF-8 expression appeared to be widespread  
10 although the expression levels varied among individual muscles.

#### EXAMPLE 7

#### CHROMOSOMAL LOCALIZATION OF GDF-8

- In order to map the chromosomal location of GDF-8, DNA samples from human/rodent somatic cell hybrids (Drwinga, *et al.*, *Genomics*, 16:311-413, 1993; Dubois and Naylor,  
15 *Genomics*, 16:315-319, 1993) were analyzed by polymerase chain reaction followed by Southern blotting. Polymerase chain reaction was carried out using primer #83, 5'-C-GCGGATCCGTGGATCTAAATGAGAACAGTGAGC-3' (SEQ ID NO: 15) and primer #84, 5'-CGCGAATTCTCAGGTAATGATTGTTTCCGTTGTAGCG-3' (SEQ ID NO:16) for 40 cycles at 94°C for 2 minutes, 60°C for 1 minute, and 72°C for 2 minutes. These  
20 primers correspond to nucleotides 119 to 143 (flanked by a Bam H1 recognition sequence), and nucleotides 394 to 418 (flanked by an Eco R1 recognition sequence), respectively, in the human GDF-8 cDNA sequence. PCR products were electrophoresed on agarose gels, blotted, and probed with oligonucleotide #100, 5'-ACACTAAATCTTCAAGAATA-3' (SEQ ID NO:17), which corresponds to a  
25 sequence internal to the region flanked by primer #83 and #84. Filters were hybridized in 6 X SSC, 1 X Denhardt's solution, 100µg/ml yeast transfer RNA, and 0.05% sodium pyrophosphate at 50°C.

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As shown in FIGURE 11, the human-specific probe detected a band of the predicted size (approximately 320 base pairs) in the positive control sample (total human genomic DNA) and in a single DNA sample from the human/rodent hybrid panel. This positive signal corresponds to human chromosome 2. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the lanes designated M, CHO, and H, the starting DNA template was total genomic DNA from mouse, hamster, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards. These data show that the human GDF-8 gene is located on chromosome 2.

#### EXAMPLE 8

##### GDF-8 TRANSGENIC KNOCKOUT MICE

The GDF-8, we disrupted the GDF-8 gene was disrupted by homologous targeting in embryonic stem cells. To ensure that the resulting mice would be null for GDF-8 function, the entire mature C-terminal region was deleted and replaced by a neo cassette (Figure 12a). A murine 129 SV/J genomic library was prepared in lambda FIX II according to the instructions provided by Stratagene (La Jolla, CA). The structure of the GDF-8 gene was deduced from restriction mapping and partial sequencing of phage clones isolated from this library. Vectors for preparing the targeting construct were kindly provided by Philip Soriano and Kirk Thomas University. R1 ES cells were transfected with the targeting construct, selected with gancyclovir (2  $\mu$ M) and G418 (250  $\mu$ g/ml), and analyzed by Southern analysis. Homologously targeted clones were injected into C57BL/6 blastocysts and transferred into pseudopregnant females. Germline transmission of the targeted allele was obtained in a total of 9 male chimeras from 5 independently-derived ES clones. Genomic Southern blots were hybridized at 42°C as described above and washed in 0.2X SSC, 0.1% SDS at 42°C.

For whole leg analysis, legs of 14 week old mice were skinned, treated with 0.2 M EDTA in PBS at 4°C for 4 weeks followed by 0.5 M sucrose in PBS at 4°C. For fiber number and size analysis, samples were directly mounted and frozen in isopentane as described (Brumback and Leech, *Color Atlas of Muscle Histochemistry*, pp. 9-33, PSG Publishing

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Company, Littleton, MA, 1984). Ten to 30  $\mu\text{m}$  sections were prepared using a cryostat and stained with hematoxylin and eosin. Muscle fiber numbers were determined from sections taken from the widest part of the tibialis cranialis muscle. Muscle fiber sizes were measured from photographs of sections of tibialis cranialis and gastrocnemius  
5 muscles. Fiber type analysis was carried out using the mysosin ATPase assay after pretreatment at pH 4.35 as described (Cumming, *et al.*, *Color Atlas of Muscle Pathology*, pp. 184-185, 1994) and by immunohistochemistry using an antibody directed against type I myosin (MY32, Sigma) and the Vectastain method (Vector Labs); in the immunohistochemical experiments, no staining was seen when the primary antibodies  
10 were left out. Carcasses were prepared from shaved mice by removing the all of the internal organs and associated fat and connective tissue. Fat content of carcasses from 4 month old males was determined as described (Leshner, *et al.*, *Physiol. Behavior*, 9:281, 1972).

For protein and DNA analysis, tissue was homogenized in 150 mM NaCl, 100 mM  
15 EDTA. Protein concentrations were determined using the Biorad protein assay. DNA was isolated by adding SDS to 1%, treating with 1 mg/ml proteinase K overnight at 55°C, extracting 3 times with phenol and twice with chloroform, and precipitating with ammonium acetate and EtOH. DNA was digested with 2 mg/ml RNase for 1 hour at 37°C, and following proteinase K digestion and phenol and chloroform extractions, the  
20 DNA was precipitated twice with ammonium acetate and EtOH.

Homologous targeting of the GDF-8 gene was seen in 13/131 gancyclovir/G418 doubly-resistant ES cell clones. Following injection of these targeted clones into blastocysts, we obtained chimeras from 5 independently-derived ES clones that produced heterozygous pups when crossed to C57BL/6 females (Figure 12b). Genotypic analysis  
25 of 678 offspring derived from crosses of F1 heterozygotes showed 170  $+/+$  (25%), 380  $+/-$  (56%), and 128  $-/-$  (19%). Although the ratio of genotypes was close to the expected ratio of 1:2:1, the smaller than expected number of homozygous mutants appeared to be statistically significant ( $p < 0.001$ ).

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Homozygous mutants were viable and fertile when crossed to C57BL/6 mice and to each other. Homozygous mutant animals, however, were approximately 30% larger than their heterozygous and wild type littermates (Table 1). The difference between mutant and wild type body weights appeared to be relatively constant irrespective of age and sex in adult animals. Adult mutants also displayed an abnormal body shape, with pronounced shoulders and hips. When the skin was removed from animals that had been sacrificed, it was apparent that the muscles of the mutants were much larger than those of wild type animals. The increase in skeletal muscle mass appeared to be widespread throughout the body. Individual muscles isolated from homozygous mutant animals weighed approximately 2-3 times more than those isolated from wild type littermates (Table 2). Although the magnitude of the weight increase appeared to roughly correlate with the level of GDF-8 expression in the muscles examined. To determine whether the increased muscle mass could account for the entire difference in total body weights between wild type and mutant animals or whether many tissues were generally larger in the mutants, we compared the total body weights to carcass weights. As shown in Table 3, the difference in carcass weights between wild type and mutant animals was comparable to the difference in total body weights. Moreover, because the fat content of mutant and wild type animals was similar, these data are consistent with all of the total body weight difference resulting from an increase in skeletal muscle mass, although we have not formally ruled out the possibility that differences in bone mass might also contribute to the differences in total body mass.

To determine whether the increase in skeletal muscle mass resulted from hyperplasia or from hypertrophy, histologic analysis of several different muscle groups was performed. The mutant muscle appeared grossly normal. No excess connective tissue or fat was seen nor were there any obvious signs of degeneration, such as widely varying fiber sizes (see below) or centrally-placed nuclei. Quantitation of the number of muscle fibers showed that at the widest portion of the tibialis cranialis muscle, the total cell number was 86% higher in mutant animals compared to wild type littermates [mutant = 5470 +/- 121 (n = 3), wild type = 2936 +/- 288 (n = 3);  $p < 0.01$ ]. Consistent with this result was the finding that the amount of DNA extracted from mutant muscle was roughly 50% higher



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than from wild type muscle [mutant = 350  $\mu$ g (n = 4), wild type = 233  $\mu$ g (n = 3) from pooled gastrocnemius, plantaris, triceps brachii, tibialis cranialis, and pectoralis muscles; p = 0.05]. Hence, a large part of the increase in skeletal muscle mass resulted from muscle cell hyperplasia. However, muscle fiber hypertrophy also appeared to contribute to the overall increase in muscle mass. As shown in Figure 13, the mean fiber diameter of the tibialis cranialis muscle and gastrocnemius muscle was 7% and 22% larger, respectively, in mutant animals compared to wild type littermates, suggesting that the cross-sectional area of the fibers was increased by approximately 14% and 49%, respectively. Notably, although the mean fiber diameter was larger in the mutants, the standard deviation in fiber sizes was similar between mutant and wild type muscle, consistent with the absence of muscle degeneration in mutant animals. The increase in fiber size was also consistent with the finding that the protein to DNA ratio (w/w) was slightly increased in mutant compared to wild type muscle [mutant = 871  $\pm$  111 (n = 4), wild type = 624  $\pm$  85 (n = 3); p < 0.05].

Table 4 shows a comparison between muscle weight (in grams) from wild-type (+/+), heterozygous (+/-) and a homozygous knock-out mice (-/-). The muscle mass is increased in heterozygous as compared to wild-type animals.

Finally, fiber type analysis of various muscles was carried out to determine whether the number of both type I (slow) and type II (fast) fibers was increased in the mutant animals. In most of the muscles examined, including the tibialis cranialis muscle, the vast majority of muscle fibers were type II in both mutant and wild type animals. Hence, based on the cell counts discussed above, the absolute number of type II fibers were increased in the tibialis cranialis muscle. In the soleus muscle, where the number of type I fibers was sufficiently high that we could attempt to quantitate the ratio of fiber types, the percent of type I fibers was decreased by approximately 33% in mutant compared to wild type muscle [wild type = 39.2  $\pm$  8.1 (n = 3), mutant = 26.4  $\pm$  9.3 (n = 4)]; however, the variability in this ratio for both wild type and mutant animals was too high to support any firm conclusions regarding the relative number of fiber types.

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**EXAMPLE 9****ISOLATION OF RAT AND CHICKEN GDF-8**

- In order to isolate rat and chicken GDF-8 cDNA clones, skeletal muscle cDNA libraries prepared from these species were obtained from Stratagene and screened with a murine GDF-8 probe. Library screening was carried out as described previously (Lee, Mol. Endocrinol., 4:1034-1040) except that final washes were carried out in 2 X SSC at 65°C. Partial sequence analysis of hybridizing clones revealed the presence of open reading frames highly related to murine and human GDF-8. Partial sequences of rat and chicken GDF-8 are shown in Figures 2c and 2d, respectively, and an alignment of the predicated rat and chicken GDF-8 amino acid sequences with those of murine and human GDF-8 are shown in Figure 3b. Full length rat and chicken GDF-8 is shown in Figures 14d and 14c, respectively and sequence alignment between murine, rat, human, baboon, porcine, ovine, bovine, chicken, and turkey sequences is shown in Figures 15a and 15b. All sequences contain an RSRR sequence that is likely to represent the proteolytic processing site. Following this RSRR sequence, the sequences contain a C-terminal region that is 100% conserved among all four species. The absolute conservation of the C-terminal region between species as evolutionarily far apart as humans and chickens, and baboons and turkeys, suggests that this region will be highly conserved in many other species as well.
- Similar methodology was used to obtain the nucleotide and amino acid sequences for baboon (SEQ ID NO:18 and 19, respectively; Figure 14a); bovine (SEQ ID NO:20 and 21, respectively; Figure 14b); turkey (SEQ ID NO:26 and 27, respectively; Figure 14e); porcine (SEQ ID NO:28 and 29, respectively; Figure 14f); and ovine (SEQ ID NO:30 and 31, respectively; Figure 14g).
- Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION

- (i) APPLICANT: The Johns Hopkins University School of Medicine
- (ii) TITLE OF THE INVENTION: GROWTH DIFFERENTIATION FACTOR-8
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Fish & Richardson P.C.
  - (B) STREET: 4225 Executive Square, Suite 1400
  - (C) CITY: La Jolla
  - (D) STATE: CA
  - (E) COUNTRY: US
  - (F) ZIP: 92037
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Diskette
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: Windows95
  - (D) SOFTWARE: FastSEQ for Windows Version 2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: PCT/US98/-----
  - (B) FILING DATE: 05-February-1998
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/795,071
  - (B) FILING DATE: 05-February-1997
- (viii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/847,910
  - (B) FILING DATE: 28-April-1997
- (ix) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/862,445
  - (B) FILING DATE: 23-May-1997
- (x) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Lisa A. Haile, Ph.D.
  - (B) REGISTRATION NUMBER: 38,347
  - (C) REFERENCE/DOCKET NUMBER: 07265/129W01
- (xi) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 619/678-5070
  - (B) TELEFAX: 619/678-5099

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (iii) IMMEDIATE SOURCE:

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(B) CLONE: SJL141

(ix) FEATURE:

(A) NAME/KEY: Modified Base

(B) LOCATION: 1...35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGGAATTCG GNTGGVANRA YTGGRNRTN NKCNC

35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

(B) CLONE: SJL147

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1...33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCT CANSRCARC TNCNACNRY CAT

33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

(B) CLONE: ACM13

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1...32

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGCGGATCCA GAAGTCAAGG TGACAGACAC AC

32

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

- 57 -

(vii) IMMEDIATE SOURCE:  
(B) CLONE: ACM14

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1...33  
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCGGATCCT CCTCATGAGC ACCCACAGCG GTC

33

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 550 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:  
(B) CLONE: mouse GDF-8

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 59...436  
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTAAGGTAGG AAGGATTTC	GGCTCTATTT	ACATAATTGT	TCTTTCCTTT	TCACACAG	58
AAT CCC TTT TTA GAA GTC AAG GTG ACA GAC ACA CCC AAG AGG TCC CGG	106				
Asn Pro Phe Leu Glu Val Lys Val Thr Asp Thr Pro Lys Arg Ser Arg					
1 5 10 15					
AGA GAC TTT GGG CTT GAC TGC GAT GAG CAC TCC ACG GAA TCC CGG TGC	154				
Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr Glu Ser Arg Cys					
20 25 30					
TGC CGC TAC CCC CTC ACG GTC GAT TTT GAA GCC TTT GGA TGG GAC TGG	202				
Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp					
35 40 45					
ATT ATC GCA CCC AAA AGA TAT AAG GCC AAT TAC TGC TCA GGA GAG TGT	250				
Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys					
50 55 60					
GAA TTT GTG TTT TTA CAA AAA TAT CCG CAT ACT CAT CTT GTG CAC CAA	298				
Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln					
65 70 75 80					
GCA AAC CCC AGA GGC TCA GCA GGC CCT TGC TGC ACT CCG ACA AAA ATG	346				
Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met					
85 90 95					

- 58 -

TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA TAT 394  
 Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr  
 100 105 110

GGG AAA ATT CCA GCC ATG GTA GTA GAC CGC TGT GGG TGC TCA TGAGCTTTGC 446  
 Gly Lys Ile Pro Ala Met Val Val Asp Arg Cys Gly Cys Ser  
 115 120 125

ATTAGGTTAG AAACTTCCCA AGTCATGGAA GGTCTTCCCC TCAATTTCGA AACTGTGAAT 506  
 TCCTGCAGCC CGGGGGATCC ACTAGTTCTA GAGCGGCCGC CACC 550

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asn	Pro	Phe	Leu	Glu	Val	Lys	Val	Thr	Asp	Thr	Pro	Lys	Arg	Ser	Arg
1			5					10						15	
Arg	Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His	Ser	Thr	Glu	Ser	Arg	Cys
			20					25					30		
Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu	Ala	Phe	Gly	Trp	Asp	Trp
			35				40					45			
Ile	Ile	Ala	Pro	Lys	Arg	Tyr	Lys	Ala	Asn	Tyr	Cys	Ser	Gly	Glu	Cys
			50			55					60				
Glu	Phe	Val	Phe	Leu	Gln	Lys	Tyr	Pro	His	Thr	His	Leu	Val	His	Gln
65				70					75					80	
Ala	Asn	Pro	Arg	Gly	Ser	Ala	Gly	Pro	Cys	Cys	Thr	Pro	Thr	Lys	Met
			85					90						95	
Ser	Pro	Ile	Asn	Met	Leu	Tyr	Phe	Asn	Gly	Lys	Glu	Gln	Ile	Ile	Tyr
			100					105					110		
Gly	Lys	Ile	Pro	Ala	Met	Val	Val	Asp	Arg	Cys	Gly	Cys	Ser		
			115				120					125			

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 326 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: human GDF-8

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3...326
- (D) OTHER INFORMATION:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

- 59 -

CA AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT GAT GAG CAC TCA	47
Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser	
1 5 10 15	
ACA GAA TCA CGA TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA GCT	95
Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala	
20 25 30	
TTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT AAG GCC AAT TAC	143
Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr	
35 40 45	
TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA TAT CCT CAT ACT	191
Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr	
50 55 60	
CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC CCT TGC TGT	239
His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys	
65 70 75	
ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT TTT AAT GGC AAA	287
Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys	
80 85 90 95	
GAA CAA ATA ATA TAT GGG AAA ATT CCA GCG ATG GTA GTA	326
Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val	
100 105	

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr	
1 5 10 15	
Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe	
20 25 30	
Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys	
35 40 45	
Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His	
50 55 60	
Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr	
65 70 75 80	
Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu	
85 90 95	
Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val	
100 105	

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid

- 60 -

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: SJL141
- (ix) FEATURE:  
(A) NAME/KEY: Peptide  
(B) LOCATION: 1...9  
(D) OTHER INFORMATION: "Xaa at position 3 = His, Gln, Asn, Lys, Asp, or Glu; Xaa at position 4 = Asp or Asn; Xaa at positions 6 and 7 is Val, Ile, or Met; Xaa at position 8 = Ala or Ser.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

Gly Trp Xaa Xaa Trp Xaa Xaa Xaa Pro  
1 5

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: SJL147
- (ix) FEATURE:  
(A) NAME/KEY: Peptide  
(B) LOCATION: 1...8  
(D) OTHER INFORMATION: "Xaa at position 2 = Val, Ile, Met, Thr or Ala; Xaa at position 4 = Asp or Glu; Xaa at position 7 = Gly, or Ala.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Xaa Val Xaa Ser Cys Xaa Cys  
1 5

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2676 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: Genomic DNA
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: Murine GDF-8
- (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1...2676  
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTCTCTCGGA CGGTACATGC ACTAATATTT CACTTGGCAT TACTCAAAG CAAAAGAAG



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AAATAAGAAC AAGGGAAAAA AAAAGATTGT GCTGATTTTT AAA	ATG ATG CAA AAA	115
	Met Met Gln Lys	
	1	
CTG CAA ATG TAT GTT TAT ATT TAC CTG TTC ATG CTG ATT GCT GCT GGC		163
Leu Gln Met Tyr Val Tyr Ile Tyr Leu Phe Met Leu Ile Ala Ala Gly		
5 10 15 20		
CCA GTG GAT CTA AAT GAG GGC AGT GAG AGA GAA GAA AAT GTG GAA AAA		211
Pro Val Asp Leu Asn Glu Gly Ser Glu Arg Glu Glu Asn Val Glu Lys		
25 30 35		
GAG GGG CTG TGT AAT GCA TGT GCG TGG AGA CAA AAC ACG AGG TAC TCC		259
Glu Gly Leu Cys Asn Ala Cys Ala Trp Arg Gln Asn Thr Arg Tyr Ser		
40 45 50		
AGA ATA GAA GCC ATA AAA ATT CAA ATC CTC AGT AAG CTG CGC CTG GAA		307
Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu		
55 60 65		
ACA GCT CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA CTT CTG CCA AGA		355
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Arg		
70 75 80		
GCG CCT CCA CTC CGG GAA CTG ATC GAT CAG TAC GAC GTC CAG AGG GAT		403
Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp		
85 90 95 100		
GAC AGC AGT GAT GGC TCT TTG GAA GAT GAC GAT TAT CAC GCT ACC ACG		451
Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr		
105 110 115		
GAA ACA ATC ATT ACC ATG CCT ACA GAG TCT GAC TTT CTA ATG CAA GCG		499
Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Met Gln Ala		
120 125 130		
GAT GGC AAG CCC AAA TGT TGC TTT TTT AAA TTT AGC TCT AAA ATA CAG		547
Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln		
135 140 145		
TAC AAC AAA GTA GTA AAA GCC CAA CTG TGG ATA TAT CTC AGA CCC GTC		595
Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val		
150 155 160		
AAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCC		643
Lys Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro		
165 170 175 180		
ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC		691
Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp		
185 190 195		
ATG AGC CCA GGC ACT GGT ATT TGG CAG AGT ATT GAT GTG AAG ACA GTG		739
Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val		
200 205 210		
TTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAC TTA GGC ATT GAA ATC		787
Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile		
215 220 225		
AAA GCT TTG GAT GAG AAT GGC CAT GAT CTT GCT GTA ACC TTC CCA GGA		835
Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly		
230 235 240		



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CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAA GTC AAG GTG ACA GAC	883
Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp	
245 250 255 260	
ACA CCC AAG AGG TCC CGG AGA GAC TTT GGG CTT GAC TGC GAT GAG CAC	931
Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His	
265 270 275	
TCC ACG GAA TCC CGG TGC TGC CGC TAC CCC CTC ACG GTC GAT TTT GAA	979
Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu	
280 285 290	
GCC TTT GGA TGG GAC TGG ATT ATC GCA CCC AAA AGA TAT AAG GCC AAT	1027
Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn	
295 300 305	
TAC TGC TCA GGA GAG TGT GAA TTT GTG TTT TTA CAA AAA TAT CCG CAT	1075
Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His	
310 315 320	
ACT CAT CTT GTG CAC CAA GCA AAC CCC AGA GGC TCA GCA GGC CCT TGC	1123
Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys	
325 330 335 340	
TGC ACT CCG ACA AAA ATG TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC	1171
Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly	
345 350 355	
AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCC ATG GTA GTA GAC CGC	1219
Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg	
360 365 370	
TGT GGG TGC TCA TGAGCTTTGC ATTAGGTTAG AAACCTCCCA AGTCATGGAA GGTCT	1276
Cys Gly Cys Ser	
375	
TCCCCTCAAT TTCGAAACTG TGAATTCAAG CACCACAGGC TGTAGGCCTT GAGTATGCTC	1336
TAGTAACGTA AGCACAAGCT ACAGTGTATG AACTAAAAGA GAGAATAGAT GCAATGGTTG	1396
GCATTCAACC ACCAAAATAA ACCATACTAT AGGATGTTGT ATGATTTCCA GAGTTTTTGA	1456
AATAGATGGA GATCAAATTA CATTTATGTC CATATATGTA TATTACAACT ACAATCTAGG	1516
CAAGGAAGTG AGAGCACATC TTGTGGTCTG CTGAGTTAGG AGGGTATGAT TAAAAGGTAA	1576
AGTCTTATTT CCTAACAGTT TCACTTAATA TTTACAGAAG AATCTATATG TAGCCTTTGT	1636
AAAGTGTAGG ATTGTTATCA TTTAAAAACA TCATGTACAC TTATATTTGT ATTGTATACT	1696
TGGTAAGATA AAATTCCACA AAGTAGGAAT GGGGCCTCAC ATACACATTG CCATTCTCTAT	1756
TATAATTGGA CAATCCACCA CGGTGCTAAT GCAGTGCTGA ATGGCTCCTA CTGGACCTCT	1816
CGATAGAACA CTCTACAAAG TACGAGTCTC TCTCTCCCTT CCAGGTGCAT CTCCACACAC	1876
ACAGCACTAA GTGTTCAATG CATTTTCTTT AAGGAAAGAA GAATCTTTTT TTCTAGAGGT	1936
CACTTTTCAG TCAACTCTAG CACAGCGGGA GTGACTGCTG CATCTTAAAA GGCAGCCAAA	1996
CAGTATTCAT TTTTAAATCT AAATTTCAAA ATCACTGTCT GCCTTTATCA CATGGCAATT	2056
TTGTGGTAAA ATAATGGAAA TGACTGGTTC TATCAATATT GTATAAAGA CTCTGAAACA	2116
ATTACATTTA TATAATATGT ATACAATATT GTTTTGTAATA TAAGTGTCTC CTTTATATT	2176
TACTTTGGTA TATTTTTTACA CTAATGAAAT TTCAAATCAT TAAAGTACAA AGACATGTCA	2236
TGTATCACAA AAAAGGTGAC TGCTTCTATT TCAGAGTGAA TTAGCAGATT CAATAGTGGT	2296
CTTAAACTC TGTATGTTAA GATTAGAAGG TTATATTACA ATCAATTTAT GTATTTTTTA	2356
CATTATCAAC TTATGGTTTC ATGGTGGCTG TATCTATGAA TGTGGCTCCC AGTCAAATTT	2416
CAATGCCCCA CCATTTTAAA AATTACAAGC ATTACTAAAC ATACCAACAT GTATCTAAAG	2476
AAATACAAAT ATGGTATCTC AATAACAGCT ACTTTTTTAT TTTATAATTT GACAATGAAT	2536
ACATTTCTTT TATTTACTTC AGTTTTATAA ATTGGAACCT TGTTTTATCAA ATGTATTGTA	2596
CTCATAGCTA AATGAAATTA TTTCTTACAT AAAAATGTGT AGAAACTATA AATTAAAGTG	2656
TTTTTCACATT TTTGAAAGGC	2676

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 376 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Met Met Gln Lys Leu Gln Met Tyr Val Tyr Ile Tyr Leu Phe Met Leu
 1      5      10      15
Ile Ala Ala Gly Pro Val Asp Leu Asn Glu Gly Ser Glu Arg Glu Glu
 20      25      30
Asn Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Ala Trp Arg Gln Asn
 35      40      45
Thr Arg Tyr Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys
 50      55      60
Leu Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln
 65      70      75
Leu Leu Pro Arg Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp
 80      85      90
Val Gln Arg Asp Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Tyr
 100      105      110
His Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe
 115      120      125
Leu Met Gln Ala Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser
 130      135      140
Ser Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr
 145      150      155
Leu Arg Pro Val Lys Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg
 160      165      170
Leu Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser
 175      180      185
Leu Lys Leu Asp Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp
 190      195      200
Val Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu
 205      210      215
Gly Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val
 220      225      230
Thr Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val
 235      240      245
Lys Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp
 250      255      260
Cys Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr
 265      270      275
Val Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg
 280      285      290
Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln
 295      300      305
Lys Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser
 310      315      320
Ala Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu
 325      330      335
Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met
 340      345      350
Val Val Asp Arg Cys Gly Cys Ser
 355      360      365
370      375

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(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2743 base pairs

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(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: Human GDF-8

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1...2743  
 (D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGAAAAGTA AAAGGAAGAA ACAAGAACAA GAAAAAAGAT TATATTGATT TTAAAATC	58
ATG CAA AAA CTG CAA CTC TGT GTT TAT ATT TAC CTG TTT ATG CTG ATT	106
Met Gln Lys Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile	
1 5 10 15	
GTT GCT GGT CCA GTG GAT CTA AAT GAG AAC AGT GAG CAA AAA GAA AAT	154
Val Ala Gly Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn	
20 25 30	
GTG GAA AAA GAG GGG CTG TGT AAT GCA TGT ACT TGG AGA CAA AAC ACT	202
Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr	
35 40 45	
AAA TCT TCA AGA ATA GAA GCC ATT AAG ATA CAA ATC CTC AGT AAA CTT	250
Lys Ser Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu	
50 55 60	
CGT CTG GAA ACA GCT CCT AAC ATC AGC AAA GAT GTT ATA AGA CAA CTT	298
Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Val Ile Arg Gln Leu	
65 70 75 80	
TTA CCC AAA GCT CCT CCA CTC CGG GAA CTG ATT GAT CAG TAT GAT GTC	346
Leu Pro Lys Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val	
85 90 95	
CAG AGG GAT GAC AGC AGC GAT GGC TCT TTG GAA GAT GAC GAT TAT CAC	394
Gln Arg Asp Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His	
100 105 110	
GCT ACA ACG GAA ACA ATC ATT ACC ATG CCT ACA GAG TCT GAT TTT CTA	442
Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu	
115 120 125	
ATG CAA GTG GAT GGA AAA CCC AAA TGT TGC TTC TTT AAA TTT AGC TCT	490
Met Gln Val Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser	
130 135 140	
AAA ATA CAA TAC AAT AAA GTA GTA AAG GCC CAA CTA TGG ATA TAT TTG	538
Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu	
145 150 155 160	
AGA CCC GTC GAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC	586
Arg Pro Val Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu	
165 170 175	
ATC AAA CCT ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG	634
Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu	
180 185 190	

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AAA CTT GAC ATG AAC CCA GGC ACT GGT ATT TGG CAG AGC ATT GAT GTG Lys Leu Asp Met Asn Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val 195 200 205	682
AAG ACA GTG TTG CAA AAT TGG CTC AAA CAA CCT GAA TCC AAC TTA GGC Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly 210 215 220	730
ATT GAA ATA AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GCT GTA ACC Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr 225 230 235 240	778
TTC CCA GGA CCA GGA GAA GAT GGG CTG AAT CCG TTT TTA GAG GTC AAG Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys 245 250 255	826
GTA ACA GAC ACA CCA AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys 260 265 270	874
GAT GAG CAC TCA ACA GAA TCA CGA TGC TGT CGT TAC CCT CTA ACT GTG Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val 275 280 285	922
GAT TTT GAA GCT TTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr 290 295 300	970
AAG GCC AAT TAC TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys 305 310 315 320	1018
TAT CCT CAT ACT CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala 325 330 335	1066
GGC CCT TGC TGT ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr 340 345 350	1114
TTT AAT GGC AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCG ATG GTA Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val 355 360 365	1162
GTA GAC CGC TGT GGG TGC TCA TGAGATTAT ATTAAGCGTT CATAACTTCC TAAAAC Val Asp Arg Cys Gly Cys Ser 370 375	1219
ATGGAAGGTT TTCCCCCTCAA CAATTTTGAA GCTGTGAAAT TAAGTACCAC AGGCTATAGG CCTAGAGTAT GCTACAGTCA CTTAAGCATA AGCTACAGTA TGTAAGCTAA AAGGGGGAAT ATATGCAATG GTTGGCATT T AACCATCCAA ACAAATCATA CAAGAAAGTT TTATGATTTC CAGAGTTTTT GAGCTAGAAG GAGATCAAAAT TACATTTATG TTCCTATATA TTACAACATC GGCGAGGAAA TGAAAGCGAT TCTCCTTGAG TTCTGATGAA TTAAGGAGT ATGCTTTAAA GTCTATTTCT TTAAAGTTTT GTTTAATATT TACAGAAAAA TCCACATACA GTATTGGTAA AATGCAGGAT TGTTATATAC CATCATTCGA ATCATCCTTA AACACTTGAA TTTATATTGT ATGGTAGTAT ACTTGTAAG ATAAAATTCC AAAAAATAG GGATGGTGCA GCATATGCAA TTCCATTCC TATTATAATT GACACAGTAC ATTAACAATC CATGCCAACG GTGCTAATAC GATAGGCTGA ATGCTCGAGG CTACCAGGTT TATCACATAA AAAACATTCA GTAAAATAGT AAGTTTCTCT TTTCTTCAGG TGCATTTTCC TACACCTCCA AATGAGGAAT GGATTTTCTT TAATGTAAGA AGAATCATTT TTCTAGAGGT TGGCTTTCAA TTCTGTAGCA TACTTGGAGA AACTGCATTA TCTTAAAAGG CAGTCAAATG GTGTTTGT TTATCAAAAT GTCAAAATAA CATACTTGA GAAGTATGTA ATTTTGTCTT TGGAAAATTA CAACACTGCC TTTGCAACAC TGCAGTTTTT ATGGTAAAAT AATAGAAATG ATCGACTCTA TCAATATTGT ATAAAAAGAC TGAAACAATG CATTATATA ATATGTATAC AATATTGTTT TGTAATAAG TGCTCTCTTT	1279 1339 1399 1459 1519 1579 1639 1699 1759 1819 1879 1939 1999 2059 2119 2179

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TTTATTTACT	TTGGTATATT	TTTACACTAA	GGACATTTCA	AATTAAGTAC	TAAGGCACAA	2239
AGACATGTCA	TGCATCACAG	AAAAGCAACT	ACTTATATTT	CAGAGCAAAT	TAGCAGATTA	2299
AATAGTGGTC	TTAAAACTCC	ATATGTTAAT	GATTAGATGG	TTATATTACA	ATCATTTTAT	2359
ATTTTTTAC	ATGATTAACA	TTCACCTATG	GATTCATGAT	GGCTGTATAA	AGTGAATTTG	2419
AAATTTCAAT	GGTTTACTGT	CATTGTGTTT	AAATCTCAAC	GTTCCATTAT	TTTAATACTT	2479
GCAAAAACAT	TACTAAGTAT	ACCAAAATAA	TTGACTCTAT	TATCTGAAAT	GAAGAATAAA	2539
CTGATGCTAT	CTCAACAATA	ACTGTTACTT	TTATTTTATA	ATTTGATAAT	GAATATATTT	2599
CTGCATTTAT	TTACTTCTGT	TTTGTAATTT	GGGATTTTGT	TAATCAAATT	TATTGTACTA	2659
TGACTAAATG	AAATTATTTT	TTACATCTAA	TTTGTAGAAA	CAGTATAAGT	TATATTAAAG	2719
TGTTTTTACA	TTTTTTTGAA	AGAC				2743

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 375 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met	Gln	Lys	Leu	Gln	Leu	Cys	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Leu	Ile
1				5					10					15	
Val	Ala	Gly	Pro	Val	Asp	Leu	Asn	Glu	Asn	Ser	Glu	Gln	Lys	Glu	Asn
			20					25					30		
Val	Glu	Lys	Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr
		35					40					45			
Lys	Ser	Ser	Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu
	50					55				60					
Arg	Leu	Glu	Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Val	Ile	Arg	Gln	Leu
65					70				75					80	
Leu	Pro	Lys	Ala	Pro	Pro	Leu	Arg	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val
			85					90						95	
Gln	Arg	Asp	Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His
		100					105					110			
Ala	Thr	Thr	Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu
		115					120					125			
Met	Gln	Val	Asp	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser
	130					135				140					
Lys	Ile	Gln	Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu
145					150					155				160	
Arg	Pro	Val	Glu	Thr	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu
			165					170					175		
Ile	Lys	Pro	Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu
	180						185						190		
Lys	Leu	Asp	Met	Asn	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val
	195					200					205				
Lys	Thr	Val	Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly
	210				215					220					
Ile	Glu	Ile	Lys	Ala	Leu	Asp	Glu	Asn	Gly	His	Asp	Leu	Ala	Val	Thr
225					230					235				240	
Phe	Pro	Gly	Pro	Gly	Glu	Asp	Gly	Leu	Asn	Pro	Phe	Leu	Glu	Val	Lys
			245					250						255	
Val	Thr	Asp	Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys
		260					265					270			
Asp	Glu	His	Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val
	275					280				285					
Asp	Phe	Glu	Ala	Phe	Gly	Trp	Asp	Trp	Ile	Ile	Ala	Pro	Lys	Arg	Tyr
	290				295					300					
Lys	Ala	Asn	Tyr	Cys	Ser	Gly	Glu	Cys	Glu	Phe	Val	Phe	Leu	Gln	Lys
305					310					315					

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Tyr	Pro	His	Thr	His	Leu	Val	His	Gln	Ala	Asn	Pro	Arg	Gly	Ser	Ala
				325					330					335	
Gly	Pro	Cys	Cys	Thr	Pro	Thr	Lys	Met	Ser	Pro	Ile	Asn	Met	Leu	Tyr
			340					345					350		
Phe	Asn	Gly	Lys	Glu	Gln	Ile	Ile	Tyr	Gly	Lys	Ile	Pro	Ala	Met	Val
		355					360					365			
Val	Asp	Arg	Cys	Gly	Cys	Ser									
	370					375									

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- SEQUENCE CHARACTERISTIC:
- (A) LENGTH: 34 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: #83

(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1..34  
(C) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGATCCG TGGATCTAAA TGAGAACAGT GAGC

34

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- SEQUENCE CHARACTERISTIC
- (A) LENGTH: 37 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: #84

(ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1...37  
(C) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCGAATTCT CAGGTAATGA TTGTTTCCGT TGTAGCG

37

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single



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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:  
(B) CLONE: #100

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1...20  
(C) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:  
ACACTAAATC TTCAAGAATA 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1055 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Baboon GDF-8

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 1...1055  
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

	ATG CAA AAA	9
	Met Gln Lys	
	1	
CTG CAA CTC TGT GTT TAT ATT TAC CTG TTT ATG CTG ATT GTT GCT GGT	54	
Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile Val Ala Gly		
5 10 15 20		
CCA GTG GAT CTA AAT GAG AAC AGT GAG CAA AAA GAA AAT GTG GAA AAA	95	
Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn Val Glu Lys		
25 30 35		
GAG GGG CTG TGT AAT GCA TGT ACT TGG AGA CAA AAC ACT AAA TCT TCA	140	
Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr Lys Ser Ser		
40 45 50		
AGA ATA GAA GCC ATT AAA ATA CAA ATC CTC AGT AAA CTT CGT CTG GAA	185	
Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu		
55 60 65		
ACA GCT CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA CTT TTA CCC AAA	230	
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Lys		
70 75 80		
GCG CCT CCA CTC CGG GAA CTG ATT GAT CAG TAT GAT GTC CAG AGG GAT	275	
Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp		
85 90 95 100		

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GAC AGC AGC GAT GGC TCT TTG GAA GAT GAC GAT TAT CAC GCT ACA ACG Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr 105 110 115	320
GAA ACA ATC ATT ACC ATG CCT ACA GAG TCT GAT TTT TTA ATG CAA GTG Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Met Gln Val 120 125 130	365
GAT GGA AAA CCC AAA TGT TGC TTC TTT AAA TTT AGC TCT AAA ATA CAA Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln 135 140 145	410
TAC AAT AAA GTG GTA AAG GCC CAA CTA TGG ATA TAT TTG AGA CCC GTC Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val 150 155 160	455
GAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCT Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro 165 170 175 180	500
ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp 185 190 195	545
ATG AAC CCA GGC ACT GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val 200 205 210	590
TTG CAA AAT TGG CTC AAA CAA CCT GAA TCC AAC TTA GGC ATT GAA ATA Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile 215 220 225	635
AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GCT GTA ACC TTC CCA GGA Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly 230 235 240	680
CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAG GTC AAG GTA ACA GAC Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp 245 250 255 260	725
ACA CCC AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT GAT GAG CAC Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His 265 270 275	770
TCA ACA GAA TCG CGA TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu 280 285 290	815
GCT CTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT AAG GCC AAT Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn 295 300 305	860
TAC TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA TAT CCT CAT Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His 310 315 320	905
ACT CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC CCT TGC Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys 325 330 335 340	950
TGT ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT TTT AAT GGC Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly 345 350 355	995

[illegible]

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 376 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Baboon GDF-8

```
(ix) FEATURE:
      (A) NAME/KEY: Protein
      (B) LOCATION: 1...376
      (D) OTHER:
```

													Met	Gln	Lys
													1		
Leu	Gln	Leu	Cys	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Leu	Ile	Val	Ala	Gly
5					10					15					20
Pro	Val	Asp	Leu	Asn	Glu	Asn	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys
				25					30						35
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser
			40					45					50		
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu
		55					60					65			
Thr	Ala	Pro	Asn	Ile	Ser	Lys	Asp	Ala	Ile	Arg	Gln	Leu	Leu	Pro	Lys
	70					75					80				
Ala	Pro	Pro	Leu	Arg	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp
85					90					95					100
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr
			105						110					115	
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Met	Gln	Val
			120					125					130		
Asp	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln
		135					140					145			
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Pro	Val
	150					155					160				
Glu	Thr	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro
165					170					175					180
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp
			185						190					195	
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val
			200					205					210		
Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly	Ile	Glu	Ile
		215					220					225			
Lys	Ala	Leu	Asp	Glu	Asn	Gly	His	Asp	Leu	Ala	Val	Thr	Phe	Pro	Gly
	230					235					240				
Pro	Gly	Glu	Asp	Gly	Leu	Asn	Pro	Phe	Leu	Glu	Val	Lys	Val	Thr	Asp
245					250					255					260
Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His
			265						270					275	
Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu
			280					285					290		

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Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn  
 295 300 305  
 Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His  
 310 315 320  
 Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys  
 325 330 335 340  
 Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly  
 345 350 355  
 Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg  
 360 365 370  
 Cys Gly Cys Ser  
 375

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1055 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: genomic DNA

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: Bovine GDF-8

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1...1055

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	ATG CAA AAA	9
	Met Gln Lys	
	1	
CTG CAA ATC TCT GTT TAT ATT TAC CTA TTT ATG CTG ATT GTT GCT GGC		54
Leu Gln Ile Ser Val Tyr Ile Tyr Leu Phe Met Leu Ile Val Ala Gly		
5 10 15 20		
CCA GTG GAT CTG AAT GAG AAC AGC GAG CAG AAG GAA AAT GTG GAA AAA		95
Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn Val Glu Lys		
25 30 35		
GAG GGG CTG TGT AAT GCA TGT TTG TGG AGG GAA AAC ACT ACA TCG TCA		140
Glu Gly Leu Cys Asn Ala Cys Leu Trp Arg Glu Asn Thr Thr Ser Ser		
40 45 50		
AGA CTA GAA GCC ATA AAA ATC CAA ATC CTC AGT AAA CTT CGC CTG GAA		185
Arg Leu Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu		
55 60 65		
ACA GCT CCT AAC ATC AGC AAA GAT GCT ATC AGA CAA CTT TTG CCC AAG		230
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Lys		
70 75 80		
GCT CCT CCA CTC CTG GAA CTG ATT GAT CAG TTC GAT GTC CAG AGA GAT		275
Ala Pro Pro Leu Leu Glu Leu Ile Asp Gln Phe Asp Val Gln Arg Asp		
85 90 95 100		
GCC AGC AGT GAC GGC TCC TTG GAA GAC GAT GAC TAC CAC GCC AGG ACG		320
Ala Ser Ser Asp Gly Ser Leu Glu Asp Asp Tyr His Ala Arg Thr		
105 110 115		

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GAA	ACG	GTC	ATT	ACC	ATG	CCC	ACG	GAG	TCT	GAT	CTT	CTA	ACG	CAA	GTG	365
Glu	Thr	Val	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Leu	Leu	Thr	Gln	Val	
		120						125				130				
GAA	GGA	AAA	CCC	AAA	TGT	TGC	TTC	TTT	AAA	TTT	AGC	TCT	AAG	ATA	CAA	410
Glu	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln	
		135					140					145				
TAC	AAT	AAA	CTA	GTA	AAG	GCC	CAA	CTG	TGG	ATA	TAT	CTG	AGG	CCT	GTC	455
Tyr	Asn	Lys	Leu	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Pro	Val	
	150					155					160					
AAG	ACT	CCT	GCG	ACA	GTG	TTT	GTG	CAA	ATC	CTG	AGA	CTC	ATC	AAA	CCC	500
Glu	Thr	Pro	Thr	Ala	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro	
165					170					175					180	
ATG	AAA	GAC	GGT	ACA	AGG	TAT	ACT	GGA	ATC	CGA	TCT	CTG	AAA	CTT	GAC	545
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp	
			185					190						195		
ATG	AAC	CCA	GGC	ACT	GGT	ATT	TGG	CAG	AGC	ATT	GAT	GTG	AAG	ACA	GTG	590
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val	
		200						205				210				
TTG	CAG	AAC	TGG	CTC	AAA	CAA	CCT	GAA	TCC	AAC	TTA	GGC	ATT	GAA	ATC	635
Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly	Ile	Glu	Ile	
	215						220					225				
AAA	GCT	TTA	GAT	GAG	AAT	GGC	CAT	GAT	CTT	GCT	GTA	ACC	TTC	CCA	GAA	680
Lys	Ala	Leu	Asp	Glu	Asn	Gly	His	Asp	Leu	Ala	Val	Thr	Phe	Pro	Glu	
	230					235					240					
CCA	GGA	GAA	GAT	GGA	CTG	ACT	CCC	TTT	TTA	GAA	GTC	AAG	GTA	ACA	GAC	725
Pro	Gly	Glu	Asp	Gly	Leu	Thr	Pro	Phe	Leu	Glu	Val	Lys	Val	Thr	Asp	
245					250					255					260	
ACA	CCA	AAA	AGA	TCT	AGG	AGA	GAT	TTT	GGG	CTT	GAT	TGT	GAT	GAA	CAC	770
Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His	
			265					270						275		
TCC	ACA	GAA	TCT	CGA	TGC	TGT	CGT	TAC	CCT	CTA	ACT	GTG	GAT	TTT	GAA	815
Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu	
		280						285				290				
GCT	TTT	GGA	TGG	GAT	TGG	ATT	ATT	GCA	CCT	AAA	AGA	TAT	AAG	GCC	AAT	860
Ala	Phe	Gly	Trp	Asp	Trp	Ile	Ile	Ala	Pro	Lys	Arg	Tyr	Lys	Ala	Asn	
	295					300						305				
TAC	TGC	TCT	GGA	GAA	TGT	GAA	TTT	GTA	TTT	TTG	CAA	AAG	TAT	CCT	CAT	905
Tyr	Cys	Ser	Gly	Glu	Cys	Glu	Phe	Val	Phe	Leu	Gln	Lys	Tyr	Pro	His	
	310					315					320					
ACC	CAT	CTT	GTG	CAC	CAA	GCA	AAC	CCC	AGA	GGT	TCA	GCC	GGC	CCC	TGC	950
Thr	His	Leu	Val	His	Gln	Ala	Asn	Pro	Arg	Gly	Ser	Ala	Gly	Pro	Cys	
325					330					335					340	
TGT	ACT	CCT	ACA	AAG	ATG	TCT	CCA	ATT	AAT	ATG	CTA	TAT	TTT	AAT	GGC	995
Cys	Thr	Pro	Thr	Lys	Met	Ser	Pro	Ile	Asn	Met	Leu	Tyr	Phe	Asn	Gly	
			345					350						355		
GAA	GGA	CAA	ATA	ATA	TAC	GGG	AAG	ATT	CCA	GCC	ATG	GTA	GTA	GAT	CGC	1040
Lys	Glu	Gln	Ile	Ile	Tyr	Gly	Lys	Ile	Pro	Ala	Met	Val	Val	Asp	Arg	
		360						365					370			

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TGT GGG TGT TCA TGA  
Cys Gly Cys Ser  
375

1055

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

[illegible]

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Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg  
360 365 370  
Cys Gly Cys Ser  
375

(2) INFORMATION FOR SEO ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1055 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

(vii) IMMEDIATE SOURCE:

(B) CLONE: Chicken GDF-8

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1...1055

(D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

														ATG	CAA	AAG	9
														Met	Gln	Lys	
														1			
CTG	GCA	GTC	TAT	GTT	TAT	ATT	TAC	CTG	TTC	ATG	CAG	ATC	GCG	GTT	GAT	54	
Leu	Ala	Val	Tyr	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Gln	Ile	Ala	Val	Asp		
5					10					15					20		
CCG	GTG	GCT	CTG	GAT	GGC	AGT	AGT	CAG	CCC	ACA	GAG	AAC	GCT	GAA	AAA	95	
Pro	Val	Ala	Leu	Asp	Gly	Ser	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys		
				25					30					35			
GAC	GGA	CTG	TGC	AAT	GCT	TGT	ACG	TGG	AGA	CAG	AAT	ACA	AAA	TCC	TCC	140	
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser		
			40					45					50				
AGA	ATA	GAA	GCC	ATA	AAA	ATT	CAA	ATC	CTC	AGC	AAA	CTG	CGC	CTG	GAA	185	
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu		
		55					60					65					
CAA	GCA	CCT	AAC	ATT	AGC	AGG	GAC	GTT	ATT	AAG	CAG	CTT	TTA	CCC	AAA	230	
Gln	Ala	Pro	Asn	Ile	Ser	Arg	Asp	Val	Ile	Lys	Gln	Leu	Leu	Pro	Lys		
	70					75					80						
GCT	CCT	CCA	CTG	CAG	GAA	CTG	ATT	GAT	CAG	TAT	GAT	GTC	CAG	AGG	GAC	275	
Ala	Pro	Pro	Leu	Gln	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp		
85				90						95					100		
GAC	AGT	AGC	GAT	GGC	TCT	TTG	GAA	GAC	GAT	GAC	TAT	CAT	GCC	ACA	ACC	320	
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr		
				105					110					115			
GAG	ACG	ATT	ATC	ACA	ATG	CCT	ACG	GAG	TCT	GAT	TTT	CTT	GTA	CAA	ATG	365	
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Val	Gln	Met		
			120					125					130				
GAG	GGA	AAA	CCA	AAA	TGT	TGC	TTC	TTT	AAG	TTT	AGC	TCT	AAA	ATA	CAA	410	
Glu	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln		
		135					140					145					

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TAT AAC AAA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTG AGG CAA GTC Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Gln Val 150 155 160	455
CAA AAA CCT ACA ACG GTG TTT GTG CAG ATC CTG AGA CTC ATT AAG CCC Gln Lys Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro 165 170 175 180	500
ATG AAA GAC GGT ACA AGA TAT ACT GGA ATT CGA TCT TTG AAA CTT GAC Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp 185 190 195	545
ATG AAC CCA GGC ACT GGT ATC TGG CAG AGT ATT GAT GTG AAG ACA GTG Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val 200 205 210	590
CTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAT TTA GGC ATC GAA ATA Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile 215 220 225	635
AAA GCT TTT GAT GAG ACT GGA CGA GAT CTT GCT GTC ACA TTC CCA GGA Lys Ala Phe Asp Glu Thr Gly Arg Asp Leu Ala Val Thr Phe Pro Gly 230 235 240	680
CCA GGA GAA GAT GGA TTG AAC CCA TTT TTA GAG GTC AGA GTT ACA GAC Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Arg Val Thr Asp 245 250 255 260	725
ACA CCG AAA CGG TCC CGC AGA GAT TTT GGC CTT GAC TGT GAT GAG CAC Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His 265 270 275	770
TCA ACG GAA TCC CGA TGT TGT CGC TAC CCG CTG ACA GTG GAT TTC GAA Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu 280 285 290	815
GCT TTT GGA TGG GAC TGG ATT ATA GCA CCT AAA AGA TAC AAA GCC AAT Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn 295 300 305	860
TAC TGC TCC GGA GAA TGC GAA TTT GTG TTT CTA CAG AAA TAC CCG CAC Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His 310 315 320	905
ACT CAC CTG GTA CAC CAA GCA AAT CCC AGA GGC TCA GCA GGC CCT TGC Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys 325 330 335 340	950
TGC ACA CCC ACC AAG ATG TCC CCT ATA AAC ATG CTG TAT TTC AAT GGA Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly 345 350 355	995
AAA GAA CAA ATA ATA TAT GGA AAG ATA CCA GCC ATG GTT GTA GAT CGT Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg 360 365 370	1040
TGC GGG TGC TCA TGA Cys Gly Cys Ser 375	1055

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:



(A) LENGTH: 376 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Chicken GDF-8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

														Met	Gln	Lys
Leu	Ala	Val	Tyr	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Gln	Ile	Ala	Val	Asp	
5					10					15					20	
Pro	Val	Ala	Leu	Asp	Gly	Ser	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys	
				25					30					35		
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser	
			40					45					50			
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu	
		55					60					65				
Gln	Ala	Pro	Asn	Ile	Ser	Arg	Asp	Val	Ile	Lys	Gln	Leu	Leu	Pro	Lys	
	70					75					80					
Ala	Pro	Pro	Leu	Gln	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp	
85					90					95					100	
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr	
				105					110					11		
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Val	Gln	Met	
			120					125					130			
Glu	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln	
		135					140					145				
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Arg	Gln	Val		
	150					155					160					
Gln	Lys	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro	
165					170					175					180	
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp	
				185					190					195		
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val	
			200					205					210			
Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly	Ile	Glu	Ile	
		215					220					225				
Lys	Ala	Phe	Asp	Glu	Thr	Gly	Arg	Asp	Leu	Ala	Val	Thr	Phe	Pro	Gly	
	230					235					240					
Pro	Gly	Glu	Asp	Gly	Leu	Asn	Pro	Phe	Leu	Glu	Val	Arg	Val	Thr	Asp	
245					250					255					260	
Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His	
				265					270					275		
Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu	
			280					285					290			
Ala	Phe	Gly	Trp	Asp	Trp	Ile	Ile	Ala	Pro	Lys	Arg	Tyr	Lys	Ala	Asn	
		295					300					305				
Tyr	Cys	Ser	Gly	Glu	Cys	Glu	Phe	Val	Phe	Leu	Gln	Lys	Tyr	Pro	His	
	310					315					320					
Thr	His	Leu	Val	His	Gln	Ala	Asn	Pro	Arg	Gly	Ser	Ala	Gly	Pro	Cys	
325					330					335					340	
Cys	Thr															

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Cys Gly Cys Ser  
375

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1276 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: Rat GDF-8

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
(B) LOCATION: 1...1276  
(D) OTHER:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

	ATG ATT CAA AAA	115
	Met Ile Gln Lys	
	1	
CCG CAA ATG TAT GTT TAT ATT TAC CTG TTT GTG CTG ATT GCT GCT GGC	163	
Pro Gln Met Tyr Val Tyr Ile Tyr Leu Phe Val Leu Ile Ala Ala Gly		
5 10 15 20		
CCA GTG GAT CTA AAT GAG GAC AGT GAG AGA GAG GCG AAT GTG GAA AAA	211	
Pro Val Asp Leu Asn Glu Asp Ser Glu Arg Glu Ala Asn Val Glu Lys		
25 30 35		
GAG GGG CTG TGT AAT GCG TGT GCG TGG AGA CAA AAC ACA AGG TAC TCC	259	
Glu Gly Leu Cys Asn Ala Cys Ala Trp Arg Gln Asn Thr Arg Tyr Ser		
40 45 50		
AGA ATA GAA GCC ATA AAA ATT CAA ATC CTC AGT AAA CTC CGC CTG GAA	307	
Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu		
55 60 65		
ACA GCG CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA CTT CTG CCC AGA	355	
Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Arg		
70 75 80		
GCG CCT CCA CTC CGG GAA CTG ATC GAT CAG TAC GAC GTC CAG AGG GAT	403	
Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp		
85 90 95 100		
GAC AGC AGT GAC GGC TCT TTG GAA GAT GAC GAT TAT CAC GCT ACC ACG	451	
Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr		
105 110 115		
GAA ACA ATC ATT ACC ATG CCT ACC GAG TCT GAC TTT CTA ATG CAA GCG	499	
Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Met Gln Ala		
120 125 130		
GAT GGA AAG CCC AAA TGT TGC TTT TTT AAA TTT AGC TCT AAA ATA CAG	547	
Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln		
135 140 145		

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TAC Tyr	AAC Asn	AAA Lys	GTG Val	GTA Val	AAG Lys	GCC Ala	CAG Gln	CTG Leu	TGG Trp	ATA Ile	TAT Tyr	CTG Leu	AGA Arg	GCC Ala	GTC Val	595
150						155					160					
AAG Lys	ACT Thr	CCT Pro	ACA Thr	ACA Thr	GTG Val	TTT Phe	GTG Val	CAA Gln	ATC Ile	CTG Leu	AGA Arg	CTC Leu	ATC Ile	AAA Lys	CCC Pro	643
165					170					175					180	
ATG Met	AAA Lys	GAC Asp	GGT Gly	ACA Thr	AGG Arg	TAT Tyr	ACC Thr	GGA Gly	ATC Ile	CGA Arg	TCT Ser	CTG Leu	AAA Lys	CTT Leu	GAC Asp	691
				185					190					195		
ATG Met	AGC Ser	CCA Pro	GGC Gly	ACT Thr	GGT Gly	ATT Ile	TGG Trp	CAG Gln	AGT Ser	ATT Ile	GAT Asp	GTG Val	AAG Lys	ACA Thr	GTG Val	739
			200					205				210				
TTG Leu	CAA Gln	AAT Asn	TGG Trp	CTC Leu	AAA Lys	CAG Gln	CCT Pro	GAA Glu	TCC Ser	AAC Asn	TTA Leu	GGC Gly	ATT Ile	GAA Glu	ATC Ile	787
		215					220					225				
AAA Lys	GCT Ala	TTG Leu	GAT Asp	GAG Glu	AAT Asn	GGG Gly	CAT His	GAT Asp	CTT Leu	GCT Ala	GTA Val	ACC Thr	TTC Phe	CCA Pro	GGA Gly	835
	230					235					240					
CCA Pro	GGA Gly	GAA Glu	GAT Asp	GGG Gly	CTG Leu	AAT Asn	CCC Pro	TTT Phe	TTA Leu	GAA Glu	GTC Val	AAA Lys	GTA Val	ACA Thr	GAC Asp	883
245					250					255					260	
ACA Thr	CCC Pro	AAG Lys	AGG Arg	TCC Ser	CGG Arg	AGA Arg	GAC Asp	TTT Phe	GGG Gly	CTT Leu	GAC Asp	TGC Cys	GAT Asp	GAA Glu	CAC His	931
				265					270					275		
TCC Ser	ACG Thr	GAA Glu	TCG Ser	CGG Arg	TGC Cys	TGT Cys	CGC Arg	TAC Tyr	CCC Pro	CTC Leu	ACG Thr	GTC Val	GAT Asp	TTC Phe	GAA Glu	979
			280					285				290				
GCC Ala	TTT Phe	GGA Gly	TGG Trp	GAC Asp	TGG Trp	ATT Ile	ATT Ile	GCA Ala	CCC Pro	AAA Lys	AGA Arg	TAT Tyr	AAG Lys	GCT Ala	AAT Asn	1027
		295					300					305				
TAC Tyr	TGC Cys	TCT Ser	GGA Gly	GAG Glu	TGT Cys	GAA Glu	TTT Phe	GTG Val	TTC Phe	TTA Leu	CAA Gln	AAA Lys	TAT Tyr	CCG Pro	CAT His	1075
	310					315					320					
ACT Thr	CAT His	CTT Leu	GTG Val	CAC His	CAA Gln	GCA Ala	AAC Asn	CCC Pro	AGA Arg	GGC Gly	TCG Ser	GCA Ala	GGC Gly	CCT Pro	TGC Cys	1123
325					330					335				340		
TGC Cys	ACG Thr	CCA Pro	ACA Thr	AAA Lys	ATG Met	TCT Ser	CCC Pro	ATT Ile	AAT Asn	ATG Met	CTA Leu	TAT Tyr	TTT Phe	AAT Asn	GGC Gly	1171
				345					350				355			
AAA Lys	GAA Glu	CAA Gln	ATA Ile	ATA Ile	TAT Tyr	GGG Gly	AAA Lys	ATT Ile	CCA Pro	GCC Ala	ATG Met	GTA Val	GTA Val	GAC Asp	CGG Arg	1219
			360					365					370			
TGT Cys	GGG Gly	TGC Cys	TCG Ser	TGA												1276
			375													

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(vii) IMMEDIATE SOURCE:  
(B) CLONE: Rat GDF-8

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	52
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## (2) INFORMATION FOR SEQ ID NO:26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1055 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: Turkey GDF-8

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1...1055  
 (D) OTHER:

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

	ATG CAA AAG	9
	Met Gln Lys	
	1	
CTA GCA GTC TAT GTT TAT ATT TAC CTG TTC ATG CAG ATT TTA GTT CAT	54	
Leu Ala Val Tyr Val Tyr Ile Tyr Leu Phe Met Gln Ile Leu Val His		
5 10 15 20		
CCG GTG GCT CTT GAT GGC AGT AGT CAG CCC ACA GAG AAC GCT GAA AAA	95	
Pro Val Ala Leu Asp Gly Ser Ser Glu Gln Lys Glu Asn Val Glu Lys		
25 30 35		
GAC GGA CTG TGC AAT GCT TGC ACG TGG AGA CAG AAT ACT AAA TCC TCC	140	
Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr Lys Ser Ser		
40 45 50		
AGA ATA GAA GCC ATA AAA ATT CAA ATC CTC AGC AAA CTG CGC CTG GAA	185	
Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu		
55 60 65		
CAA GCA CCT AAC ATT AGC AGG GAC GTT ATT AAA CAA CTT TTA CCC AAA	230	
Gln Ala Pro Asn Ile Ser Arg Asp Val Ile Lys Gln Leu Leu Pro Lys		
70 75 80		
GCT CCT CCG CTG CAG GAA CTG ATT GAT CAG TAT GAC GTC CAG AGA GAC	275	
Ala Pro Pro Leu Gln Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp		
85 90 95 100		
GAC AGT AGC GAT GGC TCT TTG GAA GAC GAT GAC TAT CAT GCC ACA ACC	320	
Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr		
105 110 115		
GAA ACG ATT ATC ACA ATG CCT ACG GAG TCT GAT TTT CTT GTA CAA ATG	365	
Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Val Gln Met		
120 125 130		
GAG GGA AAA CCA AAA TGT TGC TTC TTT AAG TTT AGC TCT AAA ATA CAA	410	
Glu Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln		
135 140 145		
TAT AAC AAA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTG AGG CAA GTC	455	
Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Gln Val		
150 155 160		

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CAA AAA CCT ACA ACG GTG TTT GTG CAG ATC CTG AGA CTC ATT AAA CCC Gln Lys Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro 165 170 175 180	500
ATG AAA GAC GGT ACA AGA TAT ACT GGA ATT CGA TCT TTG AAA CTT GAC Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp 185 190 195	545
ATG AAC CCA GGC ACT GGT ATC TGG CAG AGT ATT GAT GTG AAG ACA GTG Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val 200 205 210	590
TTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAT TTA GGC ATC GAA ATA Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile 215 220 225	635
AAA GCT TTT GAT GAG AAT GGA CGA GAT CTT GCT GTA ACA TTC CCA GGA Lys Ala Phe Asp Glu Asn Gly Arg Asp Leu Ala Val Thr Phe Pro Gly 230 235 240	680
CCA GGT GAA GAT GGA CTG AAC CCA TTT TTA GAG GTC AGA GTT ACA GAC Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Arg Val Thr Asp 245 250 255 260	725
ACA CCA AAA CGG TCC CGC AGA GAT TTT GGC CTT GAC TGC GAC GAG CAC Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His 265 270 275	770
TCA ACG GAA TCT CGA TGT TGT CGC TAC CCG CTG ACA GTG GAT TTT GAA Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu 280 285 290	815
GCT TTT GGA TGG GAC TGG ATT ATA GCA CCT AAA AGA TAC AAA GCC AAT Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn 295 300 305	860
TAC TGC TCT GGA GAA TGT GAA TTC GTA TTT CTA CAG AAA TAC CCG CAC Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His 310 315 320	905
ACT CAC CTG GTA CAC CAA GCA AAT CCA AGA GGC TCA GCA GGC CCT TGC Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys 325 330 335 340	950
TGC ACA CCC ACC AAG ATG TCC CCT ATA AAC ATG CTG TAT TTC AAT GGA Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly 345 350 355	995
AAA GAA CAA ATA ATA TAT GGA AAG ATA CCA GCC ATG GTT GTA GAT CGT Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg 360 365 370	1040
TGC GGG TGC TCA TGA Cys Gly Cys Ser 375	1055

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 376 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

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(vii) IMMEDIATE SOURCE:

(B) CLONE: Turkey GDF-8

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..376

(D) OTHER:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Gln Lys															
1															
Leu	Ala	Val	Tyr	Val	Tyr	Ile	Tyr	Leu	Phe	Met	Gln	Ile	Leu	Val	His
5					10					15				20	
Pro	Val	Ala	Leu	Asp	Gly	Ser	Ser	Glu	Gln	Lys	Glu	Asn	Val	Glu	Lys
				25					30					35	
Glu	Gly	Leu	Cys	Asn	Ala	Cys	Thr	Trp	Arg	Gln	Asn	Thr	Lys	Ser	Ser
			40					45					50		
Arg	Ile	Glu	Ala	Ile	Lys	Ile	Gln	Ile	Leu	Ser	Lys	Leu	Arg	Leu	Glu
	55						60					65			
Gln	Ala	Pro	Asn	Ile	Ser	Arg	Asp	Val	Ile	Lys	Gln	Leu	Leu	Pro	Lys
	70					75					80				
Ala	Pro	Pro	Leu	Gln	Glu	Leu	Ile	Asp	Gln	Tyr	Asp	Val	Gln	Arg	Asp
85					90					95				100	
Asp	Ser	Ser	Asp	Gly	Ser	Leu	Glu	Asp	Asp	Asp	Tyr	His	Ala	Thr	Thr
			105					110					115		
Glu	Thr	Ile	Ile	Thr	Met	Pro	Thr	Glu	Ser	Asp	Phe	Leu	Val	Gln	Met
			120					125					130		
Glu	Gly	Lys	Pro	Lys	Cys	Cys	Phe	Phe	Lys	Phe	Ser	Ser	Lys	Ile	Gln
		135					140					145			
Tyr	Asn	Lys	Val	Val	Lys	Ala	Gln	Leu	Trp	Ile	Tyr	Leu	Arg	Gln	Val
	150					155					160				
Gln	Lys	Pro	Thr	Thr	Val	Phe	Val	Gln	Ile	Leu	Arg	Leu	Ile	Lys	Pro
165					170				175					180	
Met	Lys	Asp	Gly	Thr	Arg	Tyr	Thr	Gly	Ile	Arg	Ser	Leu	Lys	Leu	Asp
			185					190					195		
Met	Ser	Pro	Gly	Thr	Gly	Ile	Trp	Gln	Ser	Ile	Asp	Val	Lys	Thr	Val
			200					205				210			
Leu	Gln	Asn	Trp	Leu	Lys	Gln	Pro	Glu	Ser	Asn	Leu	Gly	Ile	Glu	Ile
		215				220						225			
Lys	Ala	Phe	Asp	Glu	Asn	Gly	Arg	Asp	Leu	Ala	Val	Thr	Phe	Pro	Gly
	230					235				240					
Pro	Gly	Glu	Asp	Gly	Leu	Asn	Pro	Phe	Leu	Glu	Val	Arg	Val	Thr	Asp
245					250				255					260	
Thr	Pro	Lys	Arg	Ser	Arg	Arg	Asp	Phe	Gly	Leu	Asp	Cys	Asp	Glu	His
			265					270					275		
Ser	Thr	Glu	Ser	Arg	Cys	Cys	Arg	Tyr	Pro	Leu	Thr	Val	Asp	Phe	Glu
			280					285				290			
Ala	Phe	Gly	Trp	Asp	Trp	Ile	Ile	Ala	Pro	Lys	Arg	Tyr	Lys	Ala	Asn
	295					300						305			
Tyr	Cys	Ser	Gly	Glu	Cys	Glu	Phe	Val	Phe	Leu	Gln	Lys	Tyr	Pro	His
	310					315					320				
Thr	His	Leu	Val	His	Gln	Ala	Asn	Pro	Arg	Gly	Ser	Ala	Gly	Pro	Cys
325					330					335				340	
Cys</															

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## CLAIMS

1. A transgenic non-human animal having a transgene disrupting or interfering with expression of growth differentiation factor-8 (GDF-8) chromosomally integrated into the germ cells of the animal.
2. The transgenic animal of claim 1, wherein the animal is selected from the group of species consisting of avian, bovine, ovine, piscine, murine, and porcine.
3. The transgenic animal of claim 1 where the species is avian.
4. The transgenic animal of claim 1 where the species is bovine.
5. The transgenic animal of claim 1 where the species is porcine.
6. The transgenic animal of claim 1 where the species is ovine.
7. The transgenic animal of claim 1 where the species is piscine.
8. The transgenic animal of claim 1, wherein the transgene comprises GDF-8 antisense polynucleotide(s).
9. The transgenic animal of claim 1, wherein the transgene comprises a gene encoding a dominant negative GDF-8 polypeptide.
10. The transgenic animal of claim 1, wherein the animal is homozygous or heterozygous for GDF-8 polynucleotide.
11. A chicken or turkey egg produced by the transgenic animal of claim 3.



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12. Beef obtained from the transgenic animal of claim 4.
13. Milk obtained from the transgenic animal of claim 4.
14. Pork obtained from the transgenic animal of claim 5.
15. Lamb obtained from the transgenic animal of claim 6.
16. Chicken or turkey meat produced by the transgenic animal of claim 3.
17. A method of producing animal food products having increased muscle mass comprising:
  - a) introducing a transgene disrupting or interfering with expression of growth differentiation factor-8 (GDF-8) into an embryo into germ cells of a pronuclear embryo of the animal;
  - b) implanting the embryo into the oviduct of a pseudopregnant female thereby allowing the embryo to mature to full term progeny;
  - c) testing the progeny for presence of the transgene to identify transgene-positive progeny;
  - d) cross-breeding transgene-positive progeny to obtain further transgene-positive progeny; and
  - e) processing the progeny to obtain foodstuff.
18. The method of claim 17, wherein the transgene comprises GDF-8 antisense polynucleotides.
19. The method of claim 17, wherein the transgene comprises a gene encoding a dominant negative GDF-8 polypeptide.
20. A method of producing avian food products having reduced cholesterol levels comprising:

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- a) introducing a transgene disrupting or interfering with expression of growth differentiation factor-8 (GDF-8) into an embryo of an avian animal;
  - b) culturing the embryo under conditions whereby progeny are hatched;
  - c) testing the progeny for presence of the transgene to identify transgene-positive progeny;
  - d) cross-breeding transgene-positive progeny; and
  - e) processing the progeny to obtain foodstuff.
21. The method of claim 20, wherein the transgene comprises GDF-8 antisense polynucleotides.
22. The method of claim 20, wherein the transgene comprises a gene encoding a dominant negative GDF-8 polypeptide.
23. The transgenic animal of claim 20, wherein the transgene comprises a polynucleotide encoding a truncated GDF-8 polypeptide.
24. A method for increasing the muscle mass in an animal comprising administering to the animal an antibody, or fragment thereof, which binds to GDF-8 polypeptide.
25. The method of claim 24, wherein anti-GDF-8 antibody is administered to a domesticated animal.
26. The method of claim 24, wherein the antibody is a monoclonal antibody or a polyclonal antibody.
27. The method of claim 24, wherein the anti-GDF-8 antibody is administered by intravenous, intramuscular, multiple bolus, or subcutaneous injections.
28. The method of claim 27, wherein the anti-GDF-8 antibody is administered within a dose range of 0.1 ug/kg to 100 mg/kg.

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29. The method of claim 27, wherein the antibody is formulated in a formulation suitable for administration by injection into an animal.
30. A method of inhibiting the growth regulating actions of GDF-8 comprising contacting a GDF-8 agent with fetal or adult muscle cells or progenitor cells.
31. The method of claim 30, wherein the agent is selected from the group consisting of a monoclonal antibody, an antisense nucleic acid and a dominant negative encoding nucleic acid sequence or polypeptide.
32. The method of claim 31, wherein the antibody is a humanized monoclonal antibody or a chimeric monoclonal antibody or fragment thereof.
33. The method of claim 30, wherein the agent is administered to a patient suffering from a disorder selected from the group consisting of muscle wasting disease, neuromuscular disorder, muscle atrophy and aging.
34. The method of claim 30, wherein the agent is administered to a patient suffering from a disorder selected from the group consisting of muscular dystrophy, spinal cord injury, traumatic injury, congestive obstructive pulmonary disease (COPD), AIDS and cachexia.
35. The method of claim 30, wherein the agent is administered to a patient with muscle wasting disease or disorder by intravenous, intramuscular or subcutaneous injection.
36. The method of claim 31, wherein the monoclonal antibody is administered within a dose range between about 0.1/kg to about 100 mg/kg.
37. The method of claim 31, wherein the monoclonal antibody is formulated in a formulation suitable for administration to a patient.

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38. A method for treating a muscle or adipose tissue disorder in a subject, comprising administering a therapeutically effective amount of a GDF-8 agent to the subject, thereby inhibiting abnormal growth of muscle or adipose tissue.
39. The method as in claim 38, wherein the GDF-8 agent is selected from the group consisting of an antisense polynucleotide, a polynucleotide encoding a dominant negative GDF-8 polypeptide, a GDF-8 antibody and a polynucleotide encoding a truncated GDF-8 polypeptide.
40. The method of claim 38, wherein the disorder is a cancer selected from the group consisting of muscle, connective tissue, or bone.
41. The method of claim 38, wherein the subject has an obesity disorder.
42. A method of inhibiting the growth regulating actions of GDF-8 in a subject comprising administering to the subject, a GDF-8 agent that inhibits the action of GDF-8 in the subject.
43. The method of claim 42, wherein the GDF-8 agent is selected from the group consisting of an antisense polynucleotide, a polynucleotide encoding a dominant negative GDF-8 polypeptide, a GDF-8 antibody and a polynucleotide encoding a truncated GDF-8 polypeptide.

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44. A method for identifying a compound that affects GDF-8 activity or gene expression comprising:
- a) incubating the compound with GDF-8 polypeptide, or with a recombinant cell expressing GDF-8 under conditions sufficient to allow the components to interact; and
  - b) determining the effect of the compound on GDF-8 activity or expression.
45. The method of claim 44, wherein the effect is inhibition of GDF-8 activity or expression.
46. The method of claim 44, wherein the effect is stimulation of GDF-8 activity or expression.
47. An isolated polynucleotide encoding a truncated GDF-8 polypeptide wherein the truncation is a loss of the C-terminal active fragment of GDF-8.
48. The isolated polynucleotide of claim 47, wherein the polynucleotide is as shown in FIGURE 12a.

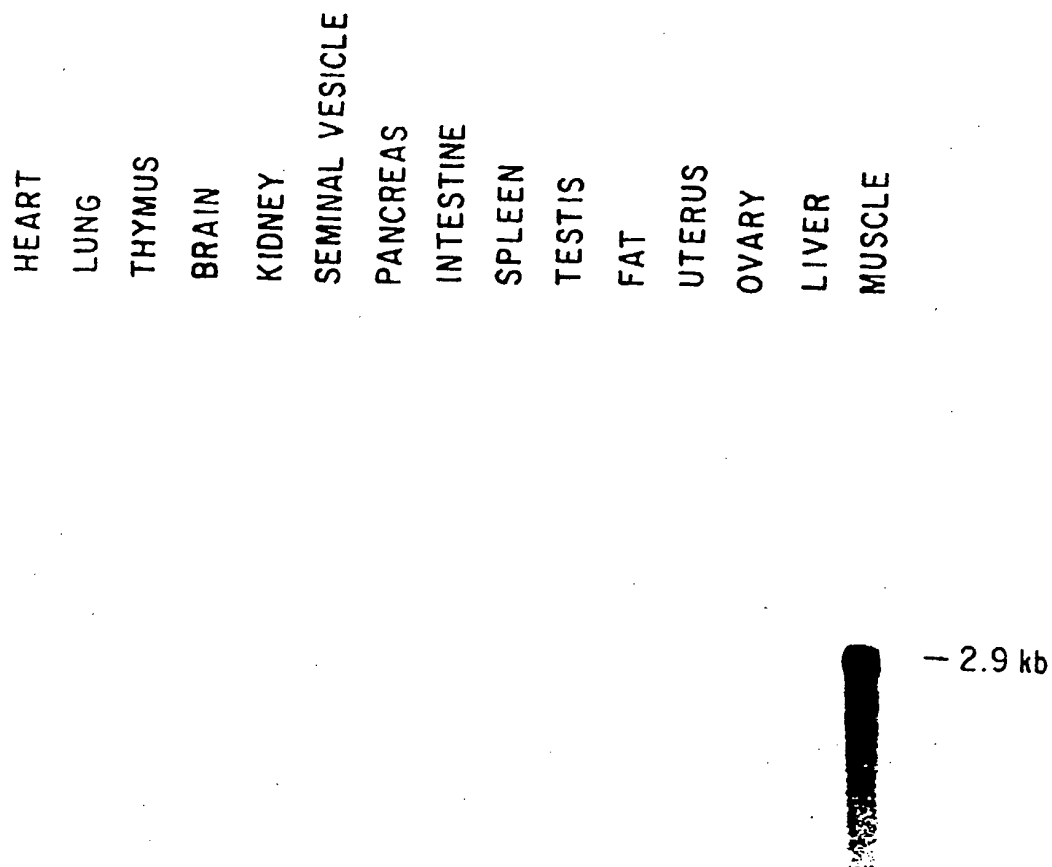


FIG. 1a

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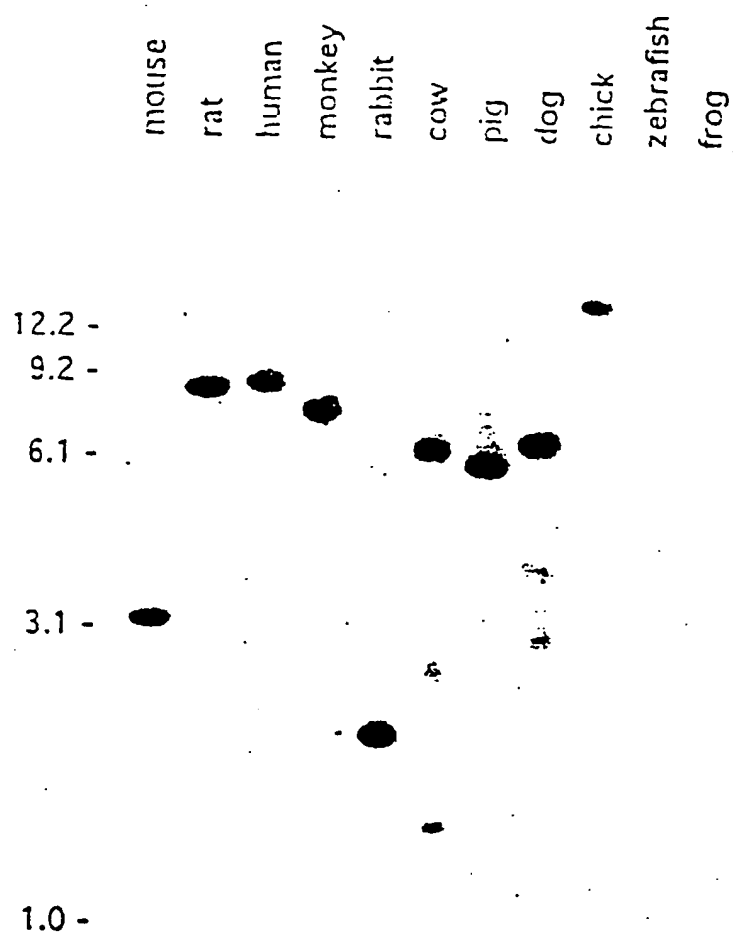


Figure 1b

1 TTAAGGTAGGAAGGATTTACGGCTCTATTTACATAATTGTTCTTTCTTTTCACACAGAA 60  
N  
61 TCCCTTTTTAGAAGTCAAGGTGACAGACACACCCAAGAGTCCCGGAGAGACTTTGGGCT 120  
P F L E V K V T D T P [K R] S [R R] D F G L  
121 TGAAGCCTTTGGATGGGACTGGATTATCGCACCCAAAAGATATAAGGCCAATTACTGCTC 180  
D C D E H S T E S R C C R Y P L T V D F  
181 TGAAGCCTTTGGATGGGACTGGATTATCGCACCCAAAAGATATAAGGCCAATTACTGCTC 240  
E A F G W D W I I A P K R Y K A N Y C S  
241 AGGAGAGTGTGAATTTGTGTTTTTACAAAATATCCGCATACTCATCTTGTGCACCAAGC 300  
G E C E F V F L Q K Y P H T H L V H Q A  
301 AAACCCAGAGGCTCAGCAGGCCCTTGCTGCACTCCGACAAAATGTCTCCCATTAATAT 360  
N P R G S A G P C C T P T K M S P I N M  
361 GCTATATTTAATGGCAAAGAACAATAATATATGGGAAAATTCAGCCATGGTAGTAGA 420  
L Y F N G K E Q I I Y C K I P A M V V D  
421 CCGCTGTGGGTGCTCATGAGCTTTGCATTAGGTTAGAAACTTCCCAAGTCATGGAAGGTC 480  
R C G C S \*  
481 TTCCCTCAATTTGAAACTGTGAATTCCTGCAGCCCGGGGATCCACTAGTTCTAGAGC 540  
541 GGCCGCCACC 550

FIG.2a

1 CAAAAGATCCAGAAGGGATTTTGGTCTTGACTGTGATGAGCACTCAACAGAATCAGCAT 60  
[K R] S [R R] D F G L D C D E H S T E S R C  
61 GCTGTCGTTACCCCTCTAACTGTGGATTTTGAAGCCTTTGGATGGGATTGGATTATCGCTC 120  
C R Y P L T V D F E A F G W D W I I A P  
121 CTAAAAGATATAAGCCAATTACTGCTCTGGAGAGTGTGAATTTGTATTTTACAAAAT 180  
K R Y K A N Y C S G E C E F V F L Q K Y  
181 ATCCTCATACTCATCTGGTACACCAAGCAAACCCAGAGGTTGAGCAGGCCCTTGCTGTA 240  
P H T H L V H Q A N P R G S A G P C C T  
241 CTCCACAAAGATGTCTCCAATTAATATGCTATATTTAATGGCAAAGAACAATAATAT 300  
P T K M S P I N M L Y F N G K E Q I I Y  
301 ATGGGAAAATTCAGCCATGGTAGTA 326  
G K I P A M V V

FIG.2b



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GAA GAT GGG CTG AAT CCC TTT TTA GAA GTC AAA GTA ACA GAC ACA CCC AAG AGG TCC CGG  
E D G L N P F L E V K V T D T P K R S R

AGA GAC TTT GGG CTT GAC TGT GAT GAA CAC TCC ACG GAA TCG CGG TGC TGT CCC TAC CCC  
R D F G L D C D E H S T E S R C C R Y P

CTC AGG GTC GAT TTT GAA GCC TTT GGA TGG GAC TGG ATT ATT GCA CCC AAA AGA TAT AAG  
L T V D F E A F G W D W I I A P K R Y K

GCT AAT TAC TGC TCT GGA GAG TGT GAA TTT GTG TTC TTA CAA AAA TAT CCG CAT ACT CAT  
A N Y C S G E C E F V F L Q K Y P H T H

CTT GTG CAC CAA GCA AAC CCC AGA GGC TCG GCA GGC CCT TGC TGC ACG CCA ACA AAA ATG  
L V H Q A N P R G S A G P C C T P T K M

TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA  
S P I N M L Y F N G K E Q I I Y G K I P

GCC ATG GTA GTA GAC CGG TGT GGG TGC TCG TGA GCT TTG CAT TAG CTT TAA AAT TTC CCA  
A M V V D R C G C S

AAT CGT GGA AGG TCT TCC CCT CGA TTT CGA AAC TGT GAA TTT ATG TAC CAC AGG CTG TAG

Rat GDF-8

FIG. 2c

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TTA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTG AGG CAA GTC CAA AAA CCT ACA ACG GTG  
L V V K A Q L W I Y L R Q V Q K P T T V  
TTT GTC CAG ATC CTG AGA CTC ATT AAG CCC ATG AAA GAC GGT ACA AGA TAT ACT GGA ATT  
F V Q I L R L I K P M K D G T R Y T G I  
GGA TCT TTG AAA CTT GAC ATG AAC CCA GGC ACT GGT ATC TGG CAG AGT ATT GAT GTG AAG  
G S L K L D M N P G T G I W Q S I D V K  
ACA GTG CTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAT TTA GGC ATC GAA ATA AAA GCT  
T V L Q N W L K Q P E S N L G I E I K A  
TTT GAT GAG ACT GGA CGA GAT CTT GCT GTC ACA TTC CCA GGA CCG GGT GAA GAT GGA TTG  
F D E T G R D L A V T F P G P G E D G L  
AAC CCA TTT TTA GAG GTC AGA GTT ACA GAC ACA CCG AAA CGG TCC CGC AGA GAT TTT GGC  
N P F L E V R V T D T P K R S R R D F G  
CTT GAC TGT GAT GAG CAC TCA ACG GAA TCC CGA TGT TGT CGC TAC CCG CTG ACA GTG GAT  
L D C D E H S T E S R C C R Y P L T V D  
TTC GAA GCT TTT GGA TGG GAC TGG ATT ATA GCA CCT AAA AGA TAC AAA GCC AAT TAC TGC  
F E A F G W D W I I A P K R Y K A N Y C  
TCC GGA GAA TGC GAA TTT GTG TTT CTA CAG AAA TAC CCG CAC ACT CAC CTG GTA CAC CAA  
S G E C E F V F L Q K Y P H T H L V H Q  
GCA AAT CCC AGA GGC TCA GCA GGC CCT TGC TGC ACA CCC ACC AAG ATG TCC CCT ATA AAC  
A N P R G S A G P C C T P T K M S P I N  
ATG CTG TAT TTC AAT GGA AAA GAA CAA ATA ATA TAT GGA AAG ATA CCA GCC ATG GTT GTA  
M L Y F N G K E Q I I Y G K I P A M V V  
GAT CGT TGC GGG TGC TCA TGA GGC TGT CGT GAG ATC CAC CAT TCG ATA AAT TGT GGA AGC  
D R C G C S  
CAC CAA AAA AAA AAG CTA TAT CCC CTC ATC CAT CTT TGA AAC TGT GAA ATT ACG TAC GCT  
AGG CAT TGC C

Chicken GDF-8

FIG. 2d

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GDF-8      SRRDFGLDCDEHSTESRCRYPLTVDF-EAFGWD-WIAPKRYKANYCSGECEVFV LQKYP—  
 GDF-1      RPRRDAEPVLGGPGGACRRRLVVSF-REVGWHRWV IAPRGFLANYCGGCCALPVALSGSGGPP  
 BMP-2      REKROAKHKQRKRLKSSCKRHPLVYDF-SDVGWNDWI VAPPGYHAFYCHGECFPLADHLNS—  
 BMP-4      KRSPKHHSQRARKKNKNCRRHSLVYDF-SDVGWNDWI VAPPGYQAFYCHGDCPFPLADHLNS—  
 Vgr-1      SRGSGSSDYNGSELKTACKKHELVSF-QDLGWQDWI IAPKGYAANYCGECFPLNAHMA—  
 OP-1      LRMANVAENSSSDQROACKKHELVSF-RDLGWQDWI IAPEGYAANYCGECFPLNSYMNA—  
 BMP-5      SRMSSVG DYNTSEKQOACKKHELVSF-RDLGWQDWI IAPEGYAANYCGECFPLNAHMA—  
 BMP-3      EQTLKKARRKQWIEPRNCARRYLVDF-ADIGWSEWI ISPKSF DAYYCSGACQFMPKSLKPS—  
 MIS      GPGRAQRSAGATAADGFCALRELSVDL—RAERSVL IPETYQANNCGVCGWPQSDRNPY—  
 Inhibin $\alpha$       ALRLLQRPPEEPAAHANCRRVALNISF-QELGWERWIVYPPSF IFHYCHGGGLHIPPNL SLPV—  
 Inhibin $\beta$ A      HRRRRRGLECDGKV-NICCKKQFFVSF-KDIGWNDWI IAPSGYHANYCGECPSH IAGTSGSSL—  
 Inhibin $\beta$ B      HRIRKRGLECDGRT-NLCCROQFFIDF-RLIGWNDWI IAPTGYGNYCEGCPAYLAGVPGSAS—  
 TGF- $\beta$ 1      HRRALDTNYCF SSTEKNCCVRQLYIDFRKDLGWK-WIHEPKGYHANFCLGCPYIWSLD—  
 TGF- $\beta$ 2      KKRALDAAYCFRNVQDNCCRLPLYIDFRKDLGWK-WIHEPKGYNANFCAGACPYLWSSD—  
 TGF- $\beta$ 3      KKRALDTNYCFRNLEENCCVRPLYIDFRQDLGWK-WIHEPKGYANFCSGCPYLRSD—

GDF-8      -HTHLVHQANPRG—SAGPCCT—PTKMSPINMLYF-NGKEQI IYGKIPAMVVDRCGCS  
 GDF-1      ALNHAVLRALMHA-AAPGAADLPCCV—PARLSPISVLFF-DNSDNVVLROYEDMVVECCGR  
 BMP-2      -TNHAI VQTLVNS—VNSKIPKACCV—PTELSAISMLYL-DENKVV LKNYQDMVVECCGR  
 BMP-4      -TNHAI VQTLVNS—VNSSIPKACCV—PTELSAISMLYL-DEYDKVVLKNYQDMVVECCGR  
 Vgr-1      -TNHAI VQTLVHL—MNP EYVPKPCA—PTKLNAISVLYF-DDNSNVILKKYRNMVVRACGH  
 OP-1      -TNHAI VQTLVHF—INPETVPKPCA—PTQLNAISVLYF-DDSSNVILKKYRNMVVRACGH  
 BMP-5      -TNHAI VQTLVHL—MFPDHVPKPCA—PTKLNAISVLYF-DDSSNVILKKYRNMVVRACGH  
 BMP-3      -NHATIQSIVRA-VGVVPGIPEPCV—PEKMSSLSILFF-DENKNVVLKVYPNMTVE SCAR  
 MIS      -GNHVVL LKMQA—RGAALARPPCCV—PTAYAGKLLISLSEER—ISAHVPMNVATECCGR  
 Inhibin $\alpha$       -PGAPPTPAQPYS—LLPGAQPCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQHCAQI  
 Inhibin $\beta$ A      -SFHSTVINHYMRGHSPFANLKSCOV—PTKL RPSMLYY-DDGQNI IKKDIQNMIVECCGS  
 Inhibin $\beta$ B      -SFHTAVVNQYRMRLNPGT-VNSCCI—PTKLSTMSMLYF-DDEYNIVKRDVPNMI VECCGA  
 TGF- $\beta$ 1      -TQYSKVLALYQ—HNPGASAAPCCV—PQALEPLPIVYY-VGRKPKV-EQLSNMIVRSCKCS  
 TGF- $\beta$ 2      -TQHSRVL SLYNT—LNPEASAPCCV—SQDLEPLTILYY-IGKTPKI-EQLSNMIVRSCKCS  
 TGF- $\beta$ 3      -TTHSTVLGLYNT—LNPEASAPCCV—PQDLEPLTILYY-VGRTPKV-EQLSNMIVRSCKCS

FIG.3a

human 1 HOKLOLCUYIYLFHLI VAGPVDL NENSE QKENVEKEGLCNACT WRONT KSSRIEAIKIOILSKRLRETAPNISKDVIRO  
murine HHOKLOMYYIYLFHLLIAAGPVDLNEGSEEREENVEKEGLCNACA WRONT RY SRIEAIKIOILSKRLRETAPNISKDAIRO  
rat chicken

human 81 LLPKAPPLRELIDQYDVQRDDSSDQSLED DDYHATTETIITHTPTESD FLHQV DQKPKCCFPKPSBKIOYNKVVUKAQLWIY  
murine LLPRAAPPLRELIDQYDVQRDDSSDQSLED DDYHATTETIITHTPTESD FLHQADGKPKCCFPKPSBKIOYNKVVUKAQLWIY  
rat chicken 160

human 161 LRPVETPTTTVFVQILRLIKPNKDGTRYTGIRSLKLDHNP GTGIWOSIDVKTVLONWLKQPE SNLGLIEIKALDENG HDLAV  
murine LRPVKETPTTTVFVQILRLIKPNKDGTRYTGIRSLKLDHNP GTGIWOSIDVKTVLONWLKQPE SNLGLIEIKALDENG HDLAV  
rat chicken LRVQVKETPTTTVFVQILRLIKPNKDGTRYTGIGSLKLDHNP GTGIWOSIDVKTVLONWLKQPE SNLGLIEIKAFDETGTGRDLAV

human 241 TFPGPGEDGLNPFLEV KVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFPVPLQ  
murine TFPGPGEDGLNPFLEV KVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFPVPLQ  
rat EDGLNPFLEV KVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFPVPLQ  
chicken TFPGPGEDGLNPFLEV KVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFPVPLQ

human 321 KYPTHLVHOANPRGSAGPCCTPTKMSPINNHL YFNGKEQIIYVKIPAMVVDRCGCS  
murine KYPTHLVHOANPRGSAGPCCTPTKMSPINNHL YFNGKEQIIYVKIPAMVVDRCGCS  
rat KYPTHLVHOANPRGSAGPCCTPTKMSPINNHL YFNGKEQIIYVKIPAMVVDRCGCS  
chicken KYPTHLVHOANPRGSAGPCCTPTKMSPINNHL YFNGKEQIIYVKIPAMVVDRCGCS

[illegible]

**FIG. 4**

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1 GTCTCTCGGACGGTACATGCACTAATATTTCACTTGGCATTACTCAAAGCAAAAAGAAG 60  
61 AAATAAGAACAAGGGAAAAAAGATTGTGCTGATTTTTAAATGATGCAAAAAGTCCA 120  
M M Q K L Q  
121 AATGTATGTTTATATTTACCTGTTTCATGCTGATTGCTGCTGGCCAGTGGATCTAAATGA 180  
M Y V Y I Y L F M L I A A G P V D L N E  
181 GGGCAGTGAGAGAGAAGAAATGTGGAAAAAGAGGGCTGTGTAATGCATGTGCGTGGAG 240  
G S E R E E N V E K E G L C N A C A W R  
241 ACAAACACGAGGTACTCCAGAATAGAAGCCATAAAATTCAAATCCTCAGTAAGCTGCG 300  
Q N T R Y S R I E A I K I Q I L S K L R  
301 CCTGGAAACAGCTCCTAACATCAGCAAAGATGCTATAAGACAACCTTCTGCAAGAGCGCC 360  
L E T A P N I S K D A I R O L L P R A P  
361 TCCACTCCGGGAAGTATGATCAGTACGACGTCCAGAGGGATGACAGCAGTATGGCTC 420  
P L R E L I D Q Y D V Q R D D S S D G S  
421 TTTGGAAGATGACGATTATCAGCTACCACGGAAACAATCATTACCATGCCTACAGAGTC 480  
L E D D D Y H A T T E T I I T M P T E S  
481 TGACTTTCTAATGCAAGCGGATGCCAAGCCCAATGTGCTTTTTTAAATTTAGCTCTAA 540  
D F L M Q A D G K P K C C F F K F S S K  
541 AATACAGTACAACAAAGTAGTAAAGCCCAACTGTGGATATATCTCAGACCCGTCAAGAC 600  
I Q Y N K V V K A Q L W I Y L R P V K T  
601 TCCTACAACAGTGTGTTGTGCAAAATCCTGAGACTCATCAAACCCATGAAAGACGGTACAAG 660  
P T T V F V Q I L R L I K P M K D G T R  
661 GTATACTGGAATCCGATCTCTGAAACTTGACATGAGCCAGGCACTGGTATTGCGCAGAG 720  
Y T G I R S L K L D M S P G T G I W Q S  
721 TATTGATGTGAAGACAGTGTGCAAAATGGCTCAAACAGCCTGAATCCAACCTAGGCAT 780  
I D V K T V L Q N W L K Q P E S N L G I  
781 TGAATCAAAGCTTTGGATGAGAATGCCATGATCTTGCTGTAACCTTCCAGGACCAGG 840  
E I K A L D E N G H D L A V T F P G P G  
841 AGAAGATGGGCTGAATCCCTTTTAGAAGTCAAGGTGACAGACACACCAAGAGGTCCCC 900  
E D G L N P F L E V K V T D T P K R S R  
901 GAGAGACTTTGGGCTTGACTGCGATGAGCACTCCACGGAATCCCGGTGCTGCGCTACCC 960  
R D F G L D C D E H S T E S R C C R Y P  
961 CCTCAGGTGATTTTGAAGCCTTTGGATGGGACTGGATTATCGCACCCAAAAGATATAA 1020  
L T V D F E A F G W D W I I A P K R Y K  
1021 GGCCAATTACTGCTCAGGAGAGTGTGAATTTGTGTTTTACAAAAATATCCGCATACTCA 1080  
A N Y C S G E C E F V F L Q K Y P H T H  
1081 TCTGTGCACCAAGCAAACCCAGAGGCTCAGCAGGCCCTTGCTGCACTCCGACAAAAAT 1140  
L V H Q A N P R G S A G P C C T P T K M  
1141 GTCTCCCATTAATATGCTATATTTAATGCCAAAGAACAATAATATATGGGAAAATTCC 1200  
S P I N M L Y F N G K E Q I I Y G K I P  
1201 AGCCATGGTAGACCGCTGTGGGTGCTCATGAGCTTTGCATTAGGTTAGAACTTCCC 1260  
A M V V D R C G C S .

FIG.5a

1261 AAGTCATGGAAGGTCTTCCCCTCAATTTGAAACTGTGAATTCAAGCACCACAGGCTGTA 1320  
1321 GGCCTTGAGTATGCTCTAGTAACGTAAGCACAAGCTACAGTGTATGAACTAAAAGAGAGA 1380  
1381 ATAGATGCAATGGTTGGCATTCAACCACCAAAATAAACCATACTATAGGATGTTGTATGA 1440  
1441 TTTCCAGAGTTTTTGAAATAGATGGAGATCAAATTACATTTATGTCCATATATGTATATT 1500  
1501 ACAACTACAATCTAGGCAAGGAAGTGAGAGCACATCTTGTGGTCTGCTGAGTTAGGAGGG 1560  
1561 TATGATTAAGGTAAAGTCTTATTTCTTAACAGTTTCACTTAATATTTACAGAAGAATC 1620  
1621 TATATGTAGCCTTTGTAAAGTGTAGGATTGTTATCATTTAAAAACATCATGTACACTTAT 1680  
1681 ATTTGTATTGTATACTTGGTAAGATAAAATCCACAAAGTAGGAATGGGGCTCACATAC 1740  
1741 ACATTGCCATTCTATTATAATTGGACAATCCACCACGGTGCTAATGCCAGTGTGAATGG 1800  
1801 CTCCTACTGGACCTCTCGATAGAACACTCTACAAAGTACGAGTCTCTCTCTCCCTTCCAG 1860  
1861 GTGCATCTCCACACACACAGCACTAAGTGTTCATGCATTTTCTTTAAGGAAAGAAGAAT 1920  
1921 CTTTTTTCTAGAGGTCAACTTTCAGTCAACTCTAGCACAGCGGGACTGACTGCTGCATC 1980  
1981 TTAAGGAGCCAAACAGTATTCATTTTTTAATCTAAATTTCAAATCACTGTCTGCCT 2040  
2041 TTATCACATGGCAATTTTGTGGTAAATAATGGAAATGACTGGTTCTATCAATATTGTAT 2100  
2101 AAAAGACTCTGAAACAATTACATTTATATAATATGTATACAATATTGTTTTGTAAATAAG 2160  
2161 TGTCTCCTTTTATATTTACTTTGGTATATTTTACACTAATGAAATTTCAAATCATTAAA 2220  
2221 GTACAAAGACATGTCATGTATCACAAAAAGGTGACTGCTTCTATTTTCAAGTGAATTAG 2280  
2281 CAGATTCAATAGTGGTCTTAAACTCTGTATGTTAAGATTAGAAGTTATATTACAATCA 2340  
2341 ATTTATGTATTTTTTACATTATCACTTATGGTTTCATGGTGGCTGTATCTATGAATGTG 2400  
2401 GCTCCCACTCAAATTTCAATGCCCCACCATTTTAAAAATTACAAGCATTACTAACATAC 2460  
2461 CAACATGTATCTAAAGAAATACAAATATGGTATCTCAATAACAGCTACTTTTTTATTTTA 2520  
2521 TAATTTGACAATGAATACATTTCTTTTATTACTTCAGTTTATAAATTGGAACTTTGT 2580  
2581 TATCAAATGTATTGTACTCATAGCTAAATGAAATTATTTCTTACATAAAAATGTGTAGAA 2640  
2641 ACTATAAATTAAGTGTTTTTACATTTTGAAGGC 2676

FIG.5b

1 AAGAAAAGTAAAAGCAAGAAACAAGAACAAGAAAAAGATTATATTGATTTTAAATCAT 60  
61 GCAAAAACCTGCAACTCTGTGTTTATATTTACCTGTTTATGCTGATTGTTGCTGGTCCAGT 120  
Q K L Q L C V Y I Y L F M L I V A G P V  
121 GGATCTAAATGAGAACAGTGAGCAAAAAGAAATGTGCAAAAAGAGGGGCTGTGTAATGC 180  
D L N E N S E Q K E N V E K E G L C N A  
181 ATGTA CTGGAGACAAAACACTAAATCTTCAAGAATAGAAGCCATTAAGATACAAATCCT 240  
C T W R Q N T K S S R I E A I K I Q I L  
241 CAGTAACTTCGTCTGGAACAGCTCCTAACATCAGCAAGATGTTATAAGACAACITTT 300  
S K L R L E T A P **N I S** K D V I R Q L L  
301 ACCCAAAGCTCCTCCACTCCGGAACTGATTGATCAGTATGATGTCCAGAGGGATGACAG 360  
P K A P P L R E L I D Q Y D V Q R D D S  
361 CAGCGATGGCTCTTTGGAAGATGACGATTATCAGCTACAACGGAACAATCATTACCAT 420  
S D G S L E D D D Y H A T T E T I I T M  
421 GCCTACAGAGTCTGATTTTCTAATGCAAGTGGATGCAAAACCCAAATGTTGCTTCTTTAA 480  
P T E S D F L M Q V D G K P K C C F F K  
481 ATTTAGCTCTAAAATACAATAACAATAAAGTAGTAAAGGCCCAACTATGGATATATTGAG 540  
F S S K I Q Y N K V V K A Q L W I Y L R  
541 ACCCGTCGAGACTCCTACAACAGTGTGTTGTCAAATCCTGAGACTCATCAACCTATGAA 600  
P V E T P T T V F V Q I L R L I K P M K  
601 AGACGGTACAAGGTATACTGGAATCCGATCTCTGAAACTTGACATGAACCCAGGCACTGG 660  
D G T R Y T G I R S L K L D M N P G T G  
661 TATTTGCCAGAGCATTGATGTGAAGACAGTGTGCAAAATTGGCTCAAACAACCTGAATC 720  
I W Q S I D V K T V L Q N W L K Q P E S  
721 CAACTTAGGCATTGAAATAAAAGCTTTAGATGAGAATGGTCATGATCTTGCTGTAACCTT 780  
N L G I E I K A L D E N G H D L A V T F  
781 CCCAGGACCAGGAGAAGATGGGCTGAATCCGTTTTTAGAGGTCAAGGTAACAGACACACC 840  
P G P G E D G L N P F L E V K V T D T P  
841 AAAAAGATCCAGAAGGGATTTTGGTCTTGACTGTGATGAGCACTCAACAGAATCAGATG 900  
K **R S R R** D F G L D C D E H S T E S R C  
901 CTGTCGTTACCCTCTAACTGTGCAATTTGAAGCTTTTGGATGGGATTGGATTATCGCTCC 960  
C R Y P L T V D F E A F G W D W I I A P  
961 TAAAAGATATAAGGCCAATTACTGCTCTGGAGAGTCTGAATTTGTATTTTACAAAATA 1020  
K R Y K A N Y C S G E C E F V F L Q K Y  
1021 TCCTCATACTCATCTGGTACACCAAGCAAAACCCAGAGGTTACAGAGGCCCTTGCTGTAC 1080  
P H T H L V H Q A N P R G S A G P C C T  
1081 TCCCACAAAGATGTCTCCAATTAATATGCTATATTTAATGGCAAAGAACAATAATATA 1140  
P T K M S P I N M L Y F N G K E Q I I Y  
1141 TGGGAAAATTCCAGCGATGGTAGTAGCCGCTGTGGGTGCTCATGAGATTATATTAAGC 1200  
G K I P A M V V D R C G C S .

FIG.5c



1201 GTTCATAACTTCCTAAAACATGGAAGGTTTTCCCTCAACAATTTGAAGCTGTGAAATT 1260  
1261 AAGTACCACAGGCTATAGGCCTAGAGTATGCTACAGTCACTTAAGCATAAGCTACAGTAT 1320  
1321 GTAAACTAAAAGGGGAATATATGCAATGGTTGGCATTTAACCATCCAAACAAATCATAC 1380  
1381 AAGAAAGTTTTATGATTTCCAGAGTTTTTGAGCTAGAAGGAGATCAAATTACATTTATGT 1440  
1441 TCCTATATATTACAACATCGGCGAGGAAATGAAAGCGATTCTCCTTGAGTTCTGATGAAT 1500  
1501 TAAAGGAGTATGCTTTAAAGTCTATTTCTTTAAAGTTTGTTTAATTTACAGAAAAAT 1560  
1561 CCACATACAGTATTGGTAAATGCAGGATTGTTATATACCATCATTCCAATCATCCTTAA 1620  
1621 ACACITGAATTTATATTGTATGGTAGTATACTTGGTAAGATAAAATCCACAAAAATAGG 1680  
1681 GATGGTGCAGCATATGCAATTTCCATTCTATTATAATTGACACAGTACATTAACAATCC 1740  
1741 ATGCCAACGGTGCTAATACGATAGGCTGAATGTCTGAGGCTACCAGGTTTATCACATAAA 1800  
1801 AAACATTCAAGTAAATAGTAAGTTCTCTTTCTTCAGGTGCATTTTCTACACCTCCAA 1860  
1861 ATGAGGAATGGATTTTCTTTAATGTAAGAAGAATCATTTTCTAGAGGTTGGCTTTCAAT 1920  
1921 TCTGTAGCATACTTGGAGAACTGCATTATCTTAAAGGCAGTCAAATGGTGTGTTTTT 1980  
1981 TATCAAAATGTCAAAATAACATACTTGGAGAAGTATGTAATTTGTCTTTGGAAAATTAC 2040  
2041 AACACTGCCCTTGGCAACTGCAGTTTTATGGTAAATAATAGAAATGATCGACTCTAT 2100  
2101 CAATATTGTATAAAAAGACTGAAACAATGCATTTATATAATATGTATACAATATTGTTTT 2160  
2161 GTAAATAAGTGCTCTCTTTTTTATTTACTTTGGTATATTTTACACTAAGGACATTTCAA 2220  
2221 ATTAAGTACTAAGGCACAAAGACATGTCATGCATCACAGAAAAGCAACTACTTATATTTT 2280  
2281 AGAGCAAATTAGCAGATTAAATAGTGGTCTTAAACTCCATATGTTAATGATTAGATGGT 2340  
2341 TATATTACAATCATTTTATATTTTTTACATGATTAACTTCATTATGGATTATGATG 2400  
2401 GCTGTATAAAGTGAATTTGAAATTTCAATGGTTTACTGTCATTGTGTTTAAATCTCAACG 2460  
2461 TTCCATTATTTTAACTTGCAAAACATTACTAAGTATACCAAATAATTGACTCTATT 2520  
2521 ATCTGAAATGAAGAATAAACTGATGCTATCTCAACAATAACTGTIACTTTTATTTTATAA 2580  
2581 TTTGATAATGAATATATTTCTGCATTTATTTACTTCTGTTTTGTAAATTGGGATTTGTT 2640  
2641 AATCAAATTTATTGTACTATGACTAAATGAAATTATTTCTTACATCTAATTGTAGAAAC 2700  
2701 AGTATAAGTTATATTAAGTGTTTTTACATTTTTTTTGAAGAC 2743

FIG.5d

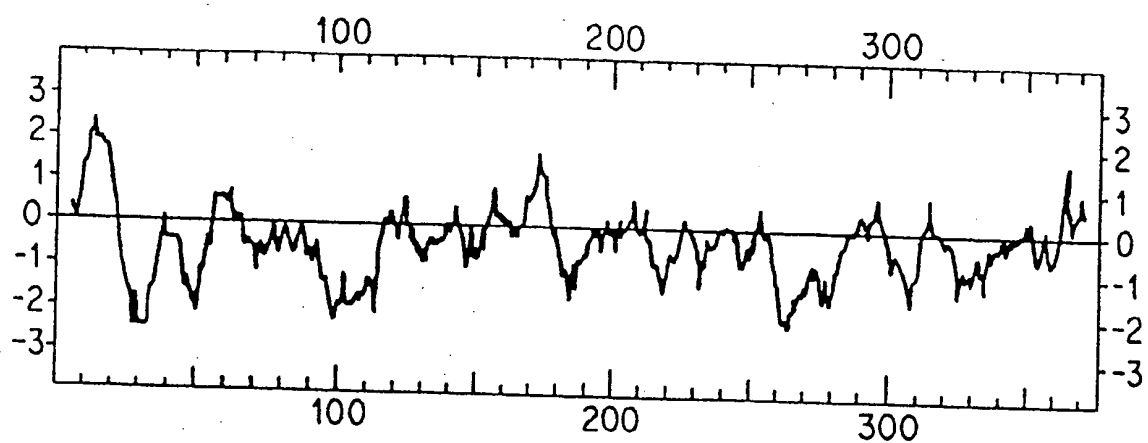


FIG. 6a

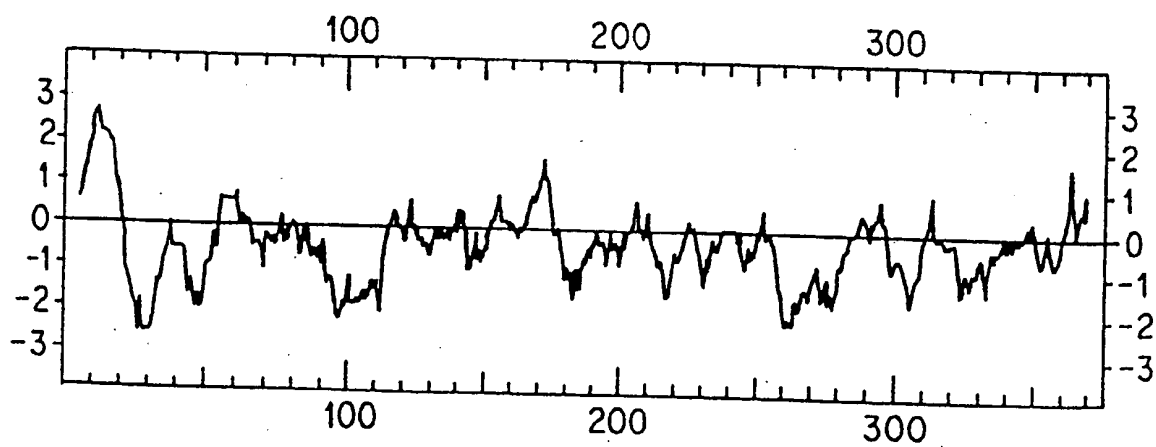
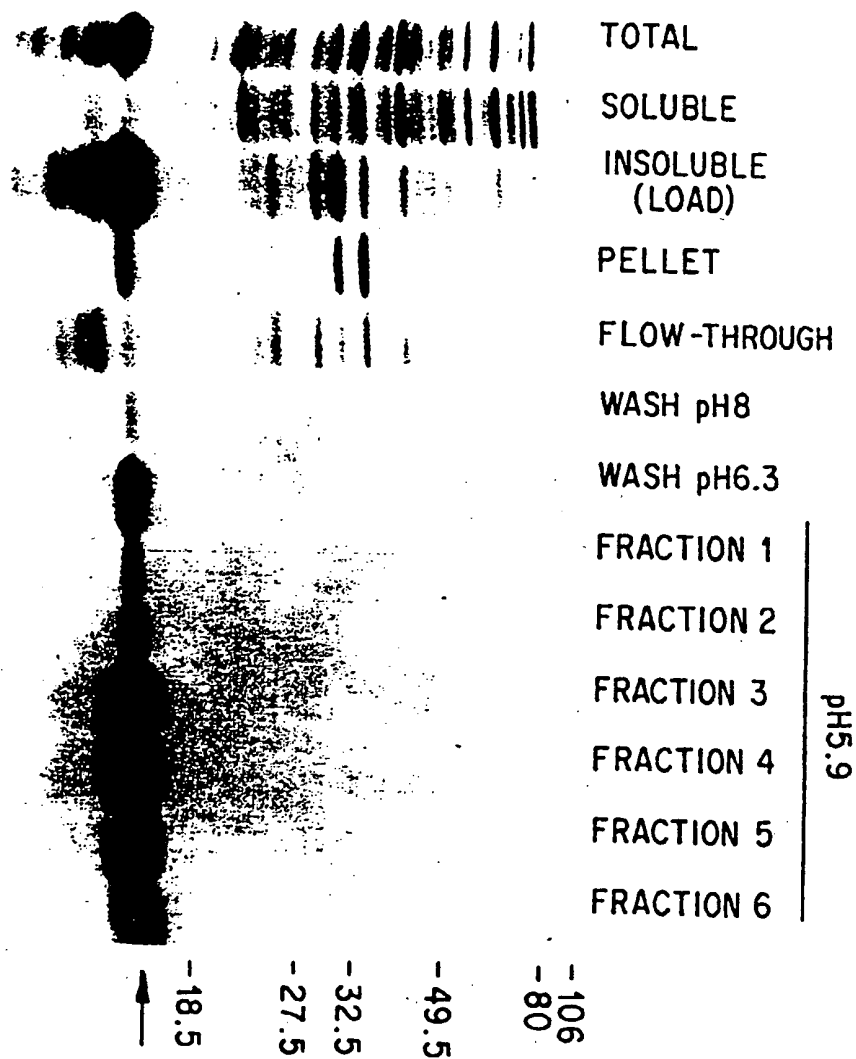


FIG. 6b

1	MMQKLMYVYIYLFMLIAAGPVDLNEGSEREENVEKEGLCNACAWRQNR	50
1	MQKLQLCVYIYLFMLIVAGPVDLNENSEQKENVEKEGLCNACTWRQNTK	49
51	YSRIEAIKIQILSKLRLETAPNISKDAIRQLLPRAAPLRELIDQYDVQRD	100
50	SSRIEAIKIQILSKLRLETAPNISKDVIRQLLPKAPLRELIDQYDVQRD	99
101	DSSDGSLEDDDYHATTETITIMPTESDFLMQADGKPKCCFFKFSSKIQYN	150
100	DSSDGSLEDDDYHATTETITIMPTESDFLMQVDGKPKCCFFKFSSKIQYN	149
151	KVVKAQLWIYLRPVKTPTTVFVQILRLIKPMKDCTRYTGIRSLKLDMSPG	200
150	KVVKAQLWIYLRPVETPTTVFVQILRLIKPMKDCTRYTGIRSLKLDMPG	199
201	TGIWQSIDVKTVLQNLKQPESNLGIEIKALDENGHD LAVTFPGPGEDGL	250
200	TGIWQSIDVKTVLQNLKQPESNLGIEIKALDENGHD LAVTFPGPGEDGL	249
251	NPFLEVKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWOWII	300
250	NPFLEVKVTDTPKRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWOWII	299
301	APKRYKANYCSGECEVFVLQKYPHTL VHQANPRGSAGPCCTPTKMSPIN	350
300	APKRYKANYCSGECEVFVLQKYPHTL VHQANPRGSAGPCCTPTKMSPIN	349
351	MLYFNGKEQIIYGKIPAMVVDRCGCS	376
350	MLYFNGKEQIIYGKIPAMVVDRCGCS	375

FIG. 7

FIG. 8



ANTISENSE SENSE

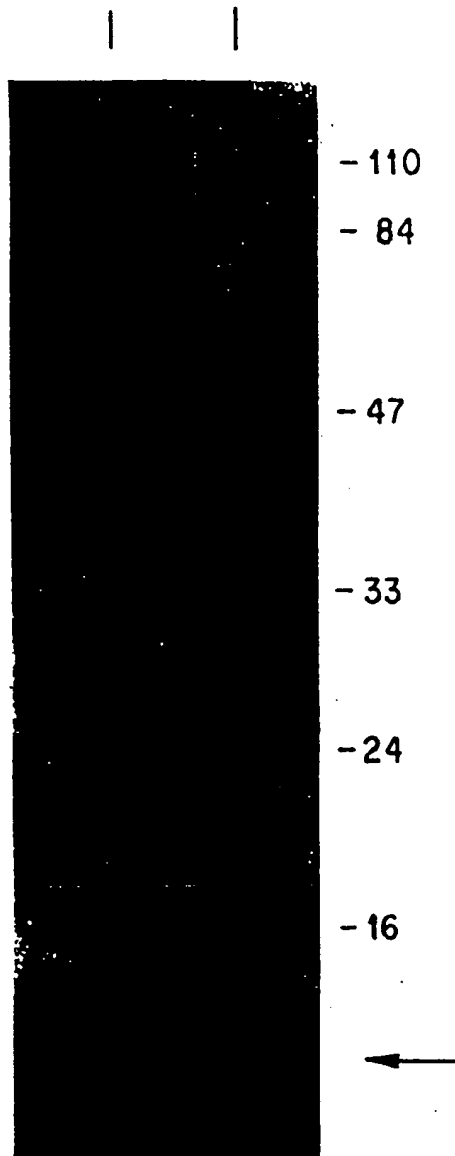


FIG. 9

FIG. 10A

HEART  
LUNG  
THYMUS  
BRAIN  
KIDNEY  
SEMINAL VESICLE  
PANCREAS  
INTESTINE  
SPLEEN  
TESTIS  
MUSCLE  
LIVER  
OVARY  
FAT  
UTERUS

— 2.9 kb

18/32

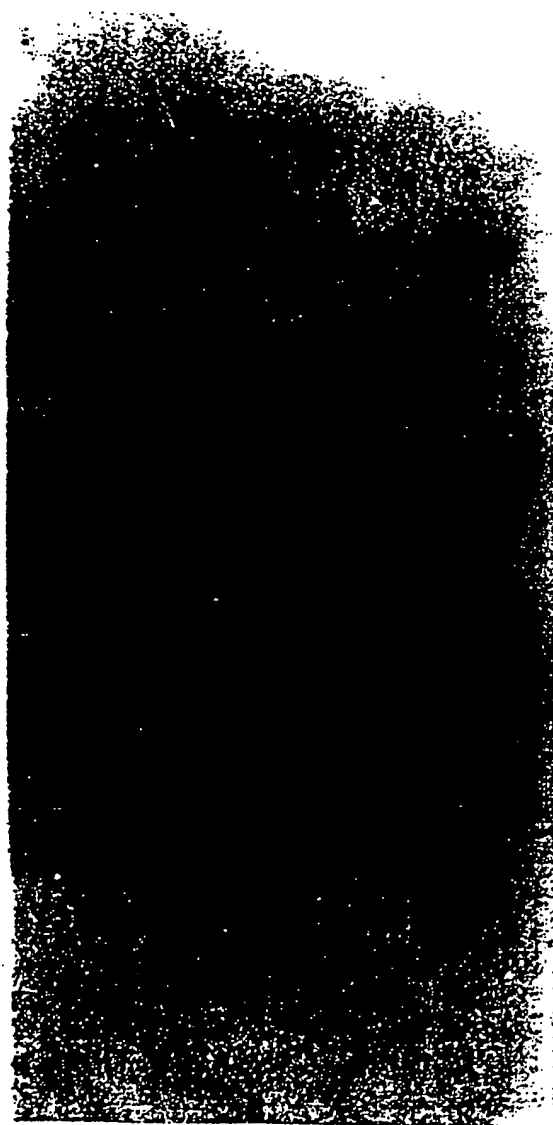
12.5 d PLACENTA

14.5 d PLACENTA

16.5 d PLACENTA

12.5 d EMBRYO

18.5 d EMBRYO



-2.9 kb

FIG. 10b

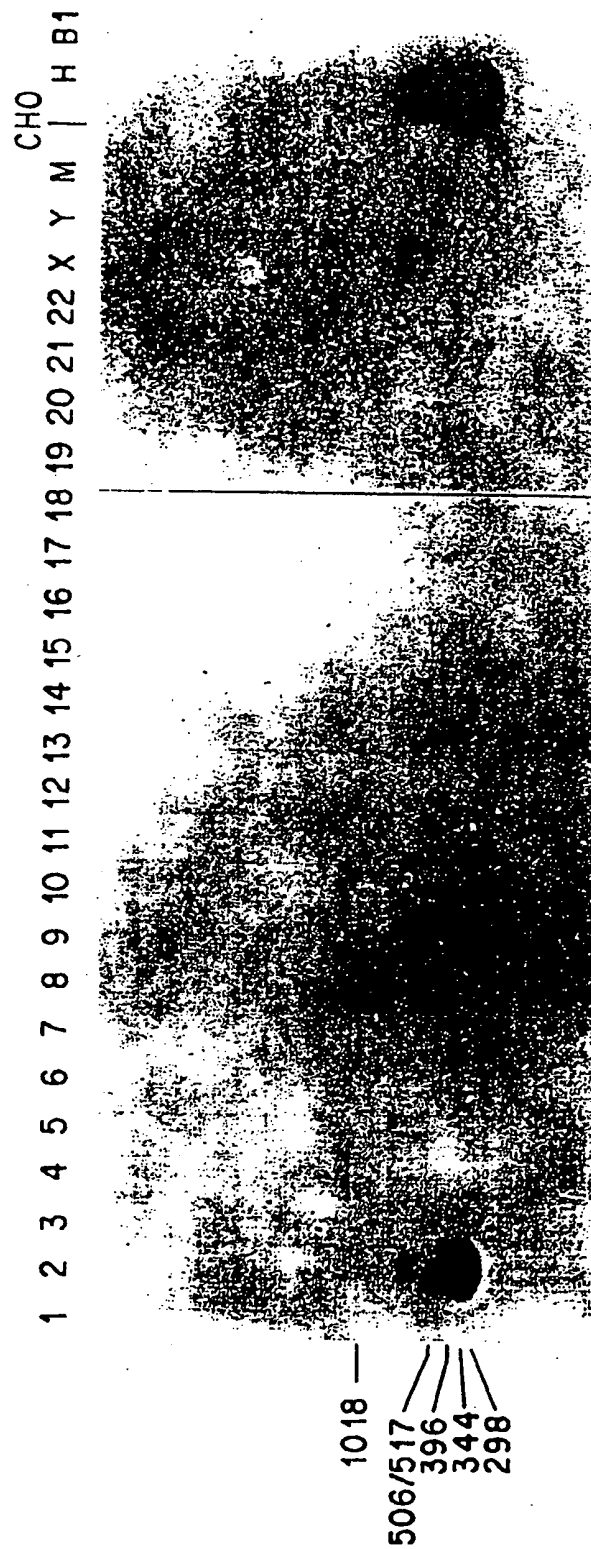


FIG.11



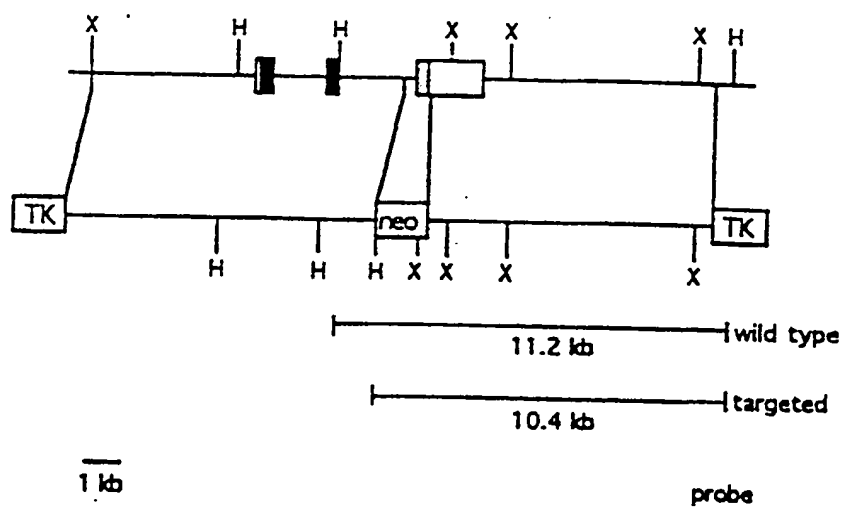


Figure 2a

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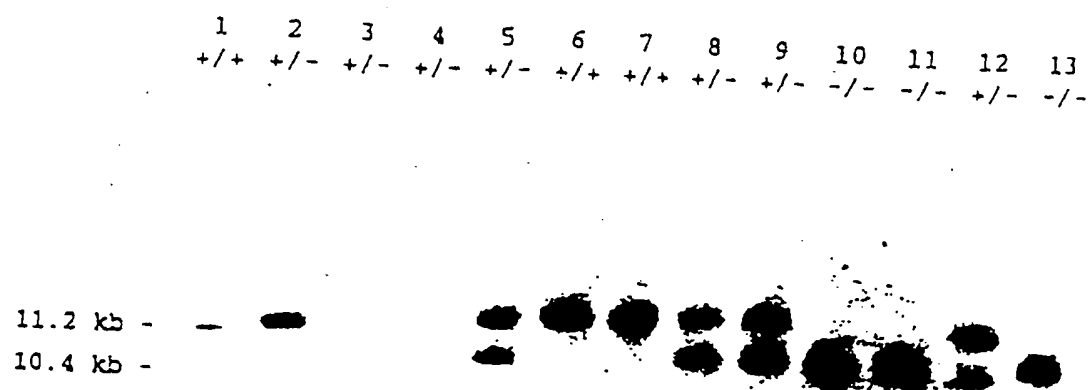


Figure 1Zb

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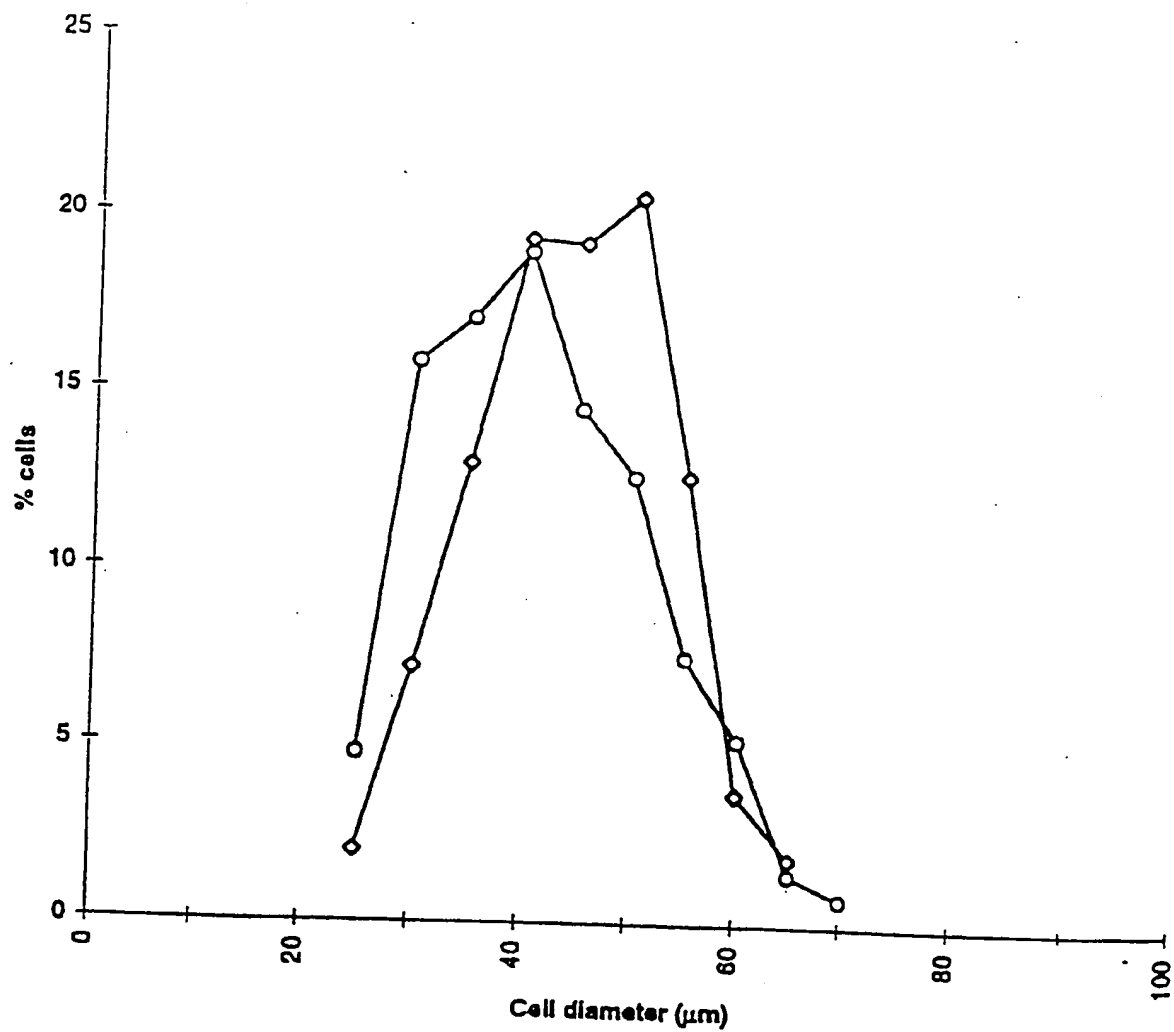


Figure 3a  
bottom

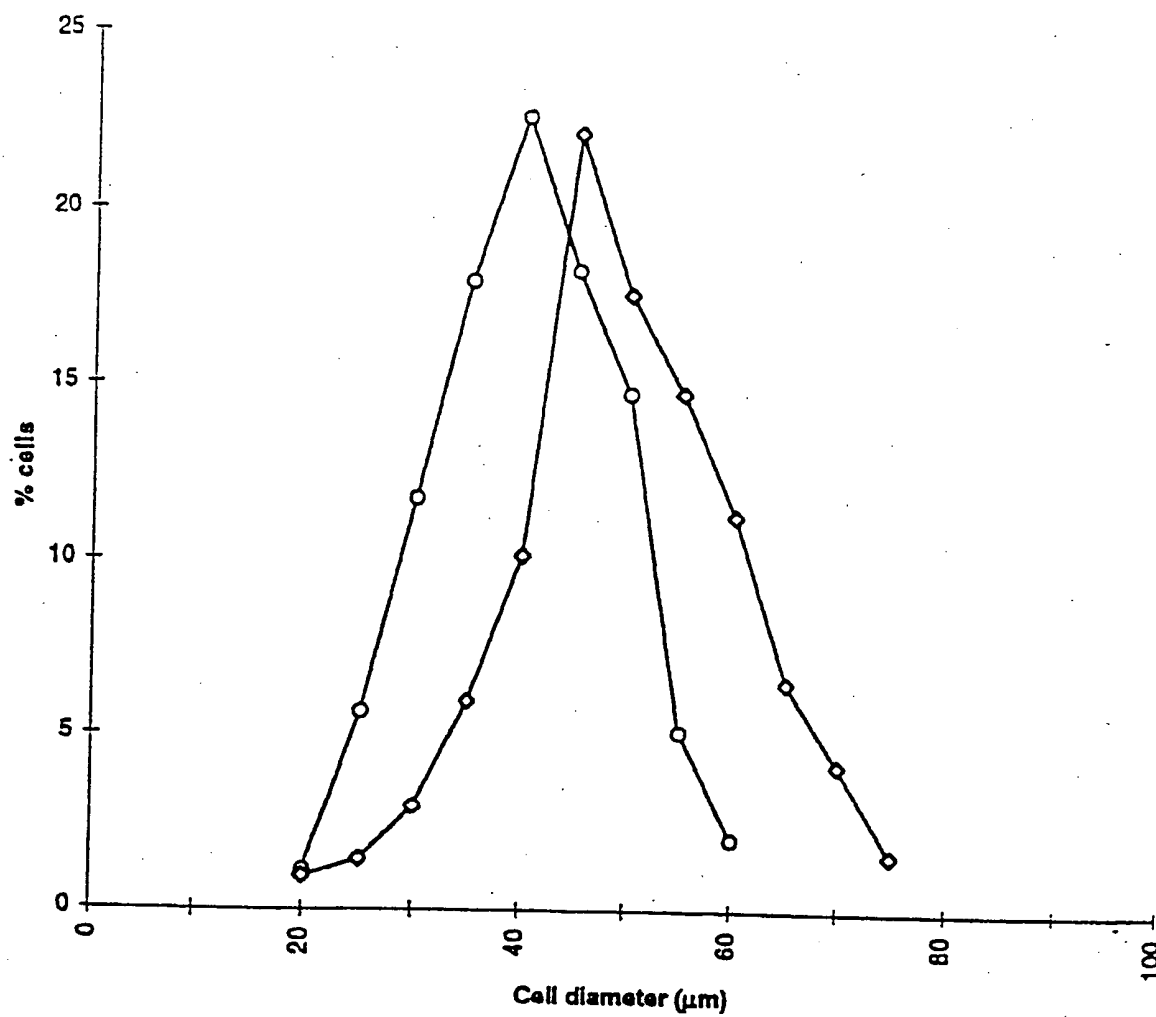


Figure 13b  
bottom

FIGURE 14a

```

1/1                               31/11
ATG CAA AAA CTC CAA CTC TGT GTT TAT ATT TAC CTG TTT ATG CTC ATT GTT GGT GGT CCA
M Q K L Q L C V Y I Y L F M L I V A C P
61/21                               91/31
GTG GAT CTA AAT GAG AAC ACT GAG CAA AAA GAA AAT GTG GAA AAA GAG GGG CTC TGT AAT
V D L N E N S E Q K E N V E K E G L C N
121/41                               151/51
GCA TGT ACT TGG AGA CAA AAC ACT AAA TCT TCA AGA ATA GAA GCC ATT AAA ATA CAA ATC
A C T W R C N T K S S R I E A I K I Q I
131/61                               211/71
CTC AGT AAA CTT COT CTC GAA ACA GCT CCT AAC ATC AGC AAA GAT GCT ATA ACA CAA CTT
L S K L R L E T A P N I S K D A I R Q L
241/81                               271/91
TTA CCC AAA GGT COT CCA CTC CGG GAA CTG ATT GAT CAG TAT GAT GTC CAG AGG GAT GAC
L P K A P P L R E L I D Q Y D V Q R D D
301/101                               331/111
AGC AGC GAT GGC TGT TGG GAA GAT GAC GAT TAT CAC GCT ACA ACC GAA ACA ATC ATT ACC
S S D G S L E D D D Y H A T T E T I I T
361/121                               391/131
ATG COT ACA GAG TGT GAT TTT TTA ATG CAA GTG GAT GGA AAA CCC AAA TGT TGC TTC TTT
M P T E S D F L M Q V D G K P K C C F F
421/141                               451/151
AAA TTT AGC TGT AAA ATA CAA TAC AAT AAA GTG GTA AAG GCC CAA CTA TGG ATA TAT TGG
K F S S K I Q Y N K V V K A Q L W I Y L
481/161                               511/171
AGA CCC CTC GAG AAT CTT ACA ACA GTC TTT GTG CAA ATC CTC ACA CTC ATC AAA COT ATG
R P V E T P T T V F V Q I L R L I K P M
541/181                               571/191
AAA GAC GGT ACA AGC TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC ATG AAC CCA GGC ACT
K D G T R Y T G I R S L K L D M N P G T
601/201                               631/211
CTT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG TTG CAA AAT TGG CTC AAA CAA COT GAA
G I W Q S I D V K T V L Q N W L K Q P E
661/221                               691/231
TCC AAC TTA GGC ATT GAA ATA AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GCT GTA ACC
S N L G I E I K A L D E N G H D L A V T
721/241                               751/251
TTC GCA GCA CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAG GTC AAG GTA ACA GAC ACA
F P G P G E D G L N P F L E V K V T D T
781/261                               811/271
CCA AAA AGA TCC AGA AGC GAT TTT GGT CTT GAC TGT GAT GAG CAC TCA ACA GAA TCG CGA
P K R S R R D F G L D C D E H S T E S R
841/281                               871/291
TGC TGT COT TAC COT CTA ACT GTG GAT TTT GAA GCT CTT GGA TGG GAT TGG ATT ATC GCT
C C R Y P L T V D F E A L G W D W I I A
901/301                               931/311
CCT AAA AGA TAT AAG GCC AAT TAC TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA
P K R Y K A N Y C S G E C E F V F L Q K
961/321                               991/331
TAT COT CAT ACT CAT CTC GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC COT TGC TGT
Y P H T H L V H Q A N P R G S A G P C C
1021/341                               1051/351
ACT CCC ACA AAG ATG TGT CCA ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA
T P T K M S P I N M L Y F N C K E Q I I
1081/361                               1111/371
TAT GCG AAA ATT CCA GCC ATG GTA GTA GAC CGC TGC GGC TGC TCA TCA
Y G K I P A M V V D R C G C S

```

FIGURE 14b

1/1 31/11  
 ATG CAA AAA CTG CAA ATC TCT GTT TAT ATT TAC CTA TTT ATG CTG ATT GTT GGT GGC CCA  
 M Q K L C I S V Y I Y L F M L I V A G P  
 61/21 91/31  
 GTG GAT CTG AAT GAG AAC AGC GAC CAG AAG GAA AAT GTG GAA AAA GAG GGC CTG TGT AAT  
 V D L N E N S E Q K E N V E K E G L C N  
 121/41 151/51  
 GCA TGT TTG TGG AGG GAA AAC ACT ACA TCC TCA AGA CTA GAA GCC ATA AAA ATC CAA ATC  
 A C L W R E N T T S S R L E A I K I Q I  
 181/61 211/71  
 CTC AGT AAA CTT GCG CTG GAA ACA GCT CCT AAC ATC AGC AAA GAT GGT ATC AGA CAA CTT  
 L S K L R L E T A P N I S K D A I R Q L  
 241/81 271/91  
 TTG CCG AAG GGT CCT CCA CTC CTG GAA CTG ATT GAT CAG TTC GAT CTC CAG AGA GAT GCC  
 L P K A F P L L E L I D Q F D V Q R D A  
 301/101 331/111  
 AGC AGT GAC GGC TCT TTG GAA GAC GAT GAC TAC CAC GCC AGG ACG GAA ACG GTC ATT ACC  
 S S D G S L E D D D Y H A R T E T V I T  
 361/121 391/131  
 ATG CCG ACG GAG TGT GAT CTT CTA ACG CAA GTG GAA GGA AAA CCG AAA TGT TCG TTC TTT  
 M P T E S D L L T Q V E G K P K C C F F  
 421/141 451/151  
 AAA TTT AGC TCT AAG ATA CAA TAC AAT AAA CTA GTA AAG GCC CAA CTG TGG ATA TAT CTG  
 K F S S K I Q Y N K L V K A Q L W I Y L  
 481/161 511/171  
 AAG CTT GTC AAG AAT CTT GCG ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCG ATC  
 R P V K T F A T V F V Q I L R L I K P M  
 541/181 571/191  
 AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC ATG AAC CCA GGC ACT  
 K D G T R Y T G I R S L K L D M N P C T  
 601/201 631/211  
 GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG TTG CAG AAC TGG CTC AAA CAA CCT GAA  
 G I W Q S I D V K T V L Q N W L K Q P E  
 661/221 691/231  
 TCC AAC TTA GCC ATT GAA ATC AAA GCT TTA GAT GAG AAT GGC CAT GAT CTT GGT GTA ACC  
 S N L G I E I K A L D E N G H D L A V T  
 721/241 751/251  
 TTC CCA GAA CCA GGA GAA GAT GGA CTG ACT CTT TTT TTA GAA GTC AAG GTA ACA GAC ACA  
 F P E P G E D G L T P F L E V K V T D T  
 781/261 811/271  
 CCA AAA AGA TGT AGG AGA GAT TTT GGG CTT GAT TGT GAT GAA CAC TCC ACA GAA TCT CGA  
 P K R S R R D F G L D C D E H S T E S R  
 841/281 871/291  
 TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA GCT TTT GGA TGG GAT TGG ATT ATT GCA  
 C C R Y P L T V D F E A F G W D W I I A  
 901/301 931/311  
 CCT AAA AGA TAT AAG GCC AAT TAC TGC TCT GGA GAA TGT GAA TTT GTA TTT TTG CAA AAG  
 P K R Y K A N Y C S G E C E F V F L Q K  
 961/321 991/331  
 TAT CCT CAT ACC CAT CTT GTG CAC CAA GCA AAC CCC AGA GGT TCA GCC GGC CCC TGC TGT  
 Y P H T H L V H Q A N P R G S A G P C C  
 1021/341 1051/351  
 ACT CCT ACA AAG ATG TCT CCA ATT AAT ATC CTA TAT TTT AAT GGC GAA GGA CAA ATA ATA  
 T P T K M S P I N M L Y F N G E G Q I I  
 1081/361 1111/371  
 TAC CCG AAG ATT CCA GCC ATG GTA GTA GAT CCG TGT GCG TGT TCA TGA  
 Y G K I P A M V V D R C G C S

FIGURE 14c

1/1  
 ATG CAA AAG CTA GCA CTC TAT GTT TAT ATT TAC CTG TTC ATG CAG ATC GCG GTT GAT CCG  
 M Q K L A V Y V Y I Y L F M Q I A V D P  
 61/21  
 GTG GGT CTG GAT GGC AGT AGT CAG CCC ACA GAG AAC GGT GAA AAA GAC GGA CTG TGC AAT  
 V A L E G S S Q P T E N A E K D G L C N  
 121/41  
 GCT TGT ACG TCG AGA CAG AAT ACA AAA TCC TCC AGA ATA GAA GCG ATA AAA ATT CAA ATC  
 A C T W R Q N T K S S R I E A I K I Q I  
 181/61  
 CTC AGC AAA CTG CCG CTG GAA CAA GCA CCT AAC ATT AGC AGG GAC GTT ATT AAG CAG CTT  
 L S K L R L E Q A P N I S R D V I K Q L  
 241/81  
 TTA CCG AAA GGT GGT CCA CTG CAG GAA CTG ATT GAT CAG TAT GAT GTC CAG AGG GAC GAC  
 L P K A P P L Q E L I D Q Y D V Q R D D  
 301/101  
 AGT AGC GAT GGC TGT TTG GAA GAC GAT GAC TAT CAT GCC ACA ACC GAG ACG ATT ATC ACA  
 S S D G S L E D D D Y H A T T E T I I T  
 361/121  
 ATG CCT ACG CAG TGT GAT TTT CTT GTA CAA ATG GAG GGA AAA CCA AAA TGT TCG TTC TTT  
 M P T E S D F L V Q M E G K P K C C F F  
 421/141  
 AAG TTT AGC TGT AAA ATA CAA TAT AAC AAA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTG  
 K F S S K I Q Y N K V V K A Q L W I Y L  
 481/161  
 AGC CAA CTC CAA AAA CTT ACA ACG GTC TTT GTG CAG ATC CTC AGA CTC ATT AAG GCG ATG  
 R Q V Q K P T T V F V Q I L R L I K P M  
 541/181  
 AAA GAC GGT ACA AGA TAT ACT GGA ATT CGA TCT TTG AAA CTT GAC ATG AAC CCA GGC ACT  
 K D G T R Y T G I R S L K L D M N P G T  
 601/201  
 GGT ATC TCG CAG AGT ATT GAT GTG AAG ACA GTG CTG CAA AAT TGG CTC AAA CAG CCT GAA  
 G I W Q S I D V K T V L Q N W L K Q P E  
 661/221  
 TCC AAT TTA GCG ATC GAA ATA AAA GCT TTT GAT GAG ACT GGA CGA GAT CTT GGT GTC ACA  
 S N L G I E I K A F D E T G R D L A V T  
 721/241  
 TTC CCA GGA CCG GGT GAA GAT GGA TTG AAC CCA TTT TTA GAG GTC AGA GTT ACA GAC ACA  
 F P G P G E D G L N P F L E V R V T D T  
 781/261  
 CCG AAA CCG TCG CCG AGA GAT TTT GCG CTT GAC TGT GAT GAG CAT TCA ACG GAA TCC CGA  
 P K R S R R D F G L D C D E H S T E S R  
 841/281  
 TGT TGT CCG TAC CCG CTG ACA GTG GAT TTC GAA GCT TTT GGA TGG GAC TGG ATT ATA GCA  
 C C R Y P L T V D F E A F G W D W I I A  
 901/301  
 CCT AAA ACA TAC AAA GCG AAT TAC TCG TCC CGA GAA TGC GAA TTT GTG TTT CTA CAG AAA  
 P K R Y K A N Y C S G E C E F V F L Q K  
 961/321  
 TAC CCG CAC ACT CAC CTG GTA CAC CAA GCA AAT CCC AGA CCG TCA CCA GCG CTT TGC TGC  
 Y P H T H L V H Q A N P R G S A G P C C  
 1021/341  
 ACA CCC ACC AAG ATG TCG CCT ATA AAC ATG CTG TAT TTC AAT GGA AAA GAA CAA ATA ATA  
 T P T K M S P I N M L Y F N G K E Q I I  
 1081/361  
 TAT GGA AAG ATA CCA GCG ATG GTT GTA GAT CGT TCG GCG TGC TCA TGA  
 Y G K I P A M V V D R C G C S \*

27/32

FIGURE 14d

1/1 31/11  
 ATG ATT CAA AAA CCG CAA ATG TAT GTT TAT ATT TAC CTG TTT GTG CTG ATT GCT CCT GCG  
 M I Q K P Q M Y V Y I Y L F V L I A A G  
 61/21 91/31  
 CCA GTC GAT CTA AAT GAG GAC AGT GAG ACA GAG GCG AAT GTG GAA AAA GAG GCG CTG TGT  
 P V D L N E D S E R E A N V E K E G L C  
 121/41 151/51  
 AAT GCG TGT GCG TCG AGA CAA AAC ACA AGC TAC TCC AGA ATA GAA CCC ATA AAA ATT CAA  
 N A C A W R Q N T R Y S R I E A I K I Q  
 191/61 211/71  
 ATC CTC AGT AAA CTC CCG CTG GAA ACA GCG CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA  
 I L S K L R L E T A P N I S K D A I R Q  
 241/81 271/91  
 CTT CTG CCG AGA GCG CCT CCA CTC CCG GAA CTG ATC GAT CAG TAC GAC CTC CAG AGC GAT  
 L L P R A P P L R E L I D Q Y D V Q R D  
 301/101 331/111  
 GAC AGC AGT GAC GCG TCT TTG GAA GAT GAC GAT TAT CAC GCT ACC ACG GAA ACA ATC ATT  
 D S S D G S L E D D D Y H A T T E T I I  
 361/121 391/131  
 ACC ATG CTT ACC GAG TCT GAC TTT CTA ATG CAA GCG GAT GGA AAG CCC AAA TGT TGC TTT  
 T M P T E S D F L M Q A D G K P K C C F  
 421/141 451/151  
 TTT AAA TTT ACC TCT AAA ATA CAG TAC AAC AAA GTG GTA AAG GCG CAG CTG TCG ATA TAT  
 F K P S S K I Q Y N K V V K A Q L W I Y  
 481/161 511/171  
 CTG AGA GCG GTC AAG ACT CTT ACA ACA GTG TTT GTG CAA ATC CTG ACA CTC ATC AAA CCG  
 L R A V K T P T T V F V Q I L R L I K P  
 541/181 571/191  
 ATG AAA GAC GGT ACA AGC TAT ACC GGA ATC CCA TCT CTG AAA CTT GAC ATG ACC CCA GCG  
 M K D G T R Y T G I R S L K L D M S P G  
 601/201 631/211  
 ACT GGT ATT TCG CAG AGT ATT GAT CTG AAG ACA CTG TTG CAA AAT TCG CTC AAA CAG CCT  
 T G I W Q S I D V K T V L Q N W L K Q P  
 661/221 691/231  
 GAA TCG AAC TTA GCG ATT GAA ATC AAA GCT TTG GAT GAG AAT GCG CAT GAT CTT GCT GTA  
 E S N L G I E I K A L D E N G H D L A V  
 721/241 751/251  
 ACC TTC CCA GGA CCA GGA GAA GAT GCG CTG AAT CCG TTT TTA GAA CTC AAA GTA ACA GAC  
 T F P G P G E D G L N P F L E V K V T D  
 781/261 811/271  
 ACA CCG AAG AGC TCG CCG AGA GAC TTT GCG CTT GAC TGT GAT GAA CAC TCG ACG GAA TCG  
 T P K R S R R D F G L D C D E H S T E S  
 841/281 871/291  
 CCG TCG TGT CCG TAC CCG CTC ACG GTC GAT TTC GAA GCG TTT GGA TCG GAC TCG ATT ATT  
 R C C R Y P L T V D F E A F G W D W I I  
 901/301 931/311  
 GCA CCG AAA AGA TAT AAG GCT AAT TAC TCG TCT GCA GAG TGT GAA TTT CTC TTC TTA CAA  
 A P K R Y K A N Y C S G E C E F V F L Q  
 961/321 991/331  
 AAA TAT CCG CAT ACT CAT CTT GTG CAC CAA GCA AAC CCG AGA GCG TCG GCA GCG CTT TCG  
 K Y P H T H L V H Q A N P R G S A G P C  
 1021/341 1051/351  
 TCG ACG CCA ACA AAA ATG TCT CCG ATT AAT ATG CTA TAT TTT AAT GCG AAA GAA CAA ATA  
 C T P T K M S P I N M L Y F N G K E Q I  
 1081/361 1111/371  
 ATA TAT CCG AAA ATT CCA CCG ATC GTA GTA CAC CCG TGT CCG TCG TCG TGA  
 I Y G K I P A M V V D R C G C S \*

Rat GDF-8



FIGURE 14e

1/1 31/11  
 ATG CAA AAG CTA GCA GTC TAT GTT TAT ATT TAC CTG TTC ATG CAG ATT TTA GTT CAT CCG  
 M Q K L A V Y V Y I Y L F M Q I L V H P  
 61/21 91/31  
 CTG GGT CTT GAT GCG AGT AGT CAG CCC ACA GAG AAC GGT GAA AAA GAC GGA CTG TGC AAT  
 V A L D G S S Q P T E N A E K D G L C N  
 121/41 151/51  
 GGT TGC ACC TGG AGA CAG AAT ACT AAA TCC TCC ACA ATA GAA GGC ATA AAA ATT CAA ATC  
 A C T W R Q N T K S S R I E A I K I Q I  
 181/61 211/71  
 CTG ACC AAA CTG GCG CTG GAA CAA GCA CTT AAC ATT AGC AGG GAC GTT ATT AAA CAA GTT  
 L S K L R L E Q A P N I S R D V I K Q L  
 241/81 271/91  
 TTA CCG AAA GGT CTT GCG CTG CAG GAA CTG ATT GAT CAG TAT CAC CTC CAG AGA GAC GAC  
 L P K A P P L Q E L I D Q Y D V Q R D D  
 301/101 331/111  
 AGT ACC GAT GCG TGT TTG GAA GAC GAT GAC TAT CAT GCG ACA ACC GAA ACC ATT ATC ACA  
 S S D G S L E D D D Y H A T T E T I I T  
 361/121 391/131  
 ATG CCG ACC GAG TGT GAT TTT CTG GTA CAA ATG GAG GGA AAA CCA AAA TGT TGC TTC TTT  
 M P T E S D F L V Q M E G K P K C C F F  
 421/141 451/151  
 AAG TTT ACC TGT AAA ACA CAA TAT AAC AAA GTA GTA AAG GCA CAA TTA TGG ATA TAC TTC  
 K F S S K I Q Y N X V V K A Q L W I Y L  
 481/161 511/171  
 AGG CAA GTC CCA AAA CTT ACA ACC GTC TTT GTC CAG ATC CTC ACA CTC ATT AAA GCG ATC  
 R C V Q R P T T V F V Q I L R L I K P M  
 541/181 571/191  
 AAA CAG CCG ACA AGA TAT ACT GCA ACT-GCA TGT TTG AAA CTT GAC ATG AAC CCA GCG ACT  
 X D G T R Y T G I R S L K L D M N F G T  
 601/201 631/211  
 GGT ATC TGG CAG AGT ATT GAT GTC AAC ACA GTC TTG CAA AAT TGG CTC AAA CAG CCG GAA  
 G I W Q S I D V K T V L Q N W L R Q P E  
 661/221 691/231  
 TCC AAT TTA GCG ATC GAA ACA AAA CCG TTT GAT GAG AAT GCA CCA GAT CTT CCG GTA ACA  
 S N L G I E I K A F D E N G R D L A V T  
 721/241 751/251  
 TTC CCA CCA CCG CAA GAT GCA CTC AAC CCA TTT TTA GAC CTC AGA GTT ACA GAC ACA  
 F P G P G E D G L N F F L E V R V T D T  
 781/261 811/271  
 CCG AAA CCG TCC CCG ACA GAT TTT GCG CTT GAC TCC GAC GAG CAC TCA ACC GAA TGT CCA  
 F K R S R R D F C L D C D E H S T E S R  
 841/281 871/291  
 TGT TGT CCG TAC CCG CTG ACA GTG GAT TTT CAA CCG TTT CCA TCG CAC TGG ATT ATA GCA  
 C C R Y P L T V D F E A P G W D W I I A  
 901/301 931/311  
 CCG AAA AGA TAC AAA GCG AAT TAC TCC TCT GCA GAA TGT CAA TTC GTA TTT CTA CAG AAA  
 P K R Y Z A N Y C S G E C E F V F L G K  
 961/321 991/331  
 TAC CCG CAC ACT CAC CTG GTA CAC CAA GCA AAT CCA AGA GCG TCA CCA GCG CCG TCC TCC  
 Y P H T H L V H Q A N P R C S A G P C C  
 1021/341 1051/351  
 ACA CCG ACC AAG ATG TCC CCG ATA AAC ATC CTG TAT TTC AAT GCA AAA CAA CAA ATA ATA  
 T P T K M S P I N M L Y F N G K E Q I I  
 1081/361 1111/371  
 TAT CCA AAG ATA CCA GCG ATG GTT GTA CAT CCG TCC CCG TCC TCA TCA  
 Y G K I P A M V V D R C C C S

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29/32

ATG CAA AAA CTG CAA ATC TAT TAT ATT TAC CTG TTT ATG CTG ATT GTT GCT GGA CCC  
 M Q K L Q I Y V Y I Y L F M L I V A G P  
 61/21 91/31  
 GTG GAT CTG AAT GAG AAC AGC GAG CAA AAG GAA AAT GTG GAA AAA GAG GGG CTG TGT AAT  
 V D L N E N S E Q K E N V E K E G L C N  
 121/41 151/51  
 GCA TGT ATG TGG AGA CAA AAC ACT AAA TCT TCA AGA CTA GAA GCC ATA AAA ATT CAA ATC  
 A C M W R Q N T K S S R L E A I K I Q I  
 181/61 211/71  
 CTC AGT AAA CTT CGC CTG GAA ACA GCT CCT AAC ATT AGC AAA GAT GCT ATA AGA CAA CTT  
 L S K L R L E T A P N I S K D A I R Q L  
 241/81 271/91  
 TTG CCC AAA GGT CCT CGA CTC CGG GAA CTG ATT GAT CAG TAC GAT GTC CAG AGA GAT GAC  
 L P K A P P L R E L I D Q Y D V Q R D D  
 301/101 331/111  
 AGC AGT GAT GGC TGG TTG GAA GAT GAT GAT TAT CAC GGT ACG ACG GAA ACG ATC ATT ACC  
 S S D G S L E D D D Y H A T T E T I I T  
 361/121 391/131  
 ATG CCT ACA GAG TCT GAT CTT CTA ATG CAA GTG GAA GGA AAA CCC AAA TGC TGC TTC TTT  
 M P T E S D L L M Q V E G K P K C C F F  
 421/141 451/151  
 AAA TTT AGC TCT AAA ATA CAA TAC AAT AAA GTA GTA AAG GCC CAA CTG TGG ATA TAT CTG  
 K F S S K I Q Y N K V V K A Q L W I Y L  
 481/161 511/171  
 AGA CCC GTC AAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCC ATG  
 R P V K T P T T V F V Q I L R L I K P M  
 541/181 571/191  
 AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC ATG AAC CCA GGC ACT  
 K D G T R Y T G I R S L K L D M N P G T  
 601/201 631/211  
 GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG TTG CAA AAT TGG CTC AAA CAA CCT GAA  
 G I W Q S I D V K T V L Q N W L K Q P E  
 661/221 691/231  
 TCC AAC TTA GGC ATT GAA ATC AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GGT GTA AGC  
 S N L G I E I K A L D E N G H D L A V T  
 721/241 751/251  
 TTC CCA GGA CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAA GTC AAG GTA ACA GAC ACA  
 F P G P G E D G L N P F L E V K V T D T  
 781/261 811/271  
 CCA AAA AGA TCC AGG AGA GAT TTT GGA CTC GAC TGT GAT GAG CAC TCA ACA GAA TCT CGA  
 P K R S R R D F G L D C D E H S T E S R  
 841/281 871/291  
 TGC TGT COT TAC CTT CTA ACT GTG GAT TTT GAA GGT TTT GGA TGG GAC TGG ATT ATT GCA  
 C C R Y P L T V D F E A F G W D W I I A  
 901/301 931/311  
 CCC AAA AGA TAT AAG GCC AAT TAC TGC TGT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA  
 P K R Y K A N Y C S G E C E F V F L Q K  
 961/321 991/331  
 TAC CTT CAC ACT CAT CTT GTG CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC CCC TGC TGT  
 Y P H T H L V H Q A N P R G S A G P C C  
 1021/341 1051/351  
 ACT CCC ACA AAG ATG TCT CCA ATC AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA  
 T P T K M S P I N M L Y F N G K E Q I I  
 1081/361 1111/371  
 TAT GGG AAA ATT CCA GCC ATG GTA GTA GAT CGC TGT GGG TGC TCA TCA  
 Y G K I P A M V V D R C G C S \*

Porcine GDF-8

FIGURE 14F

30/32

1/1  
 ATG CAA AAA CTG CAA ATC TTT GTT TAT ATT TAC CTA TTT ATG CTG CTT GTT CCT GCC CCA  
 M Q K L C I F V T I Y L F M L L V A G P  
 31/11  
 61/21  
 GTG CAT CTG AAT GAG AAC AGC GAG CAG AAG GAA AAT GTG GAA AAA AAG GGG CTG TGT AAT  
 V D L N E N S E Q K E N V E K K G L C N  
 91/31  
 121/41  
 GCA TGC TTG TGG AGA CAA AAC AAT AAA TCC TCA AGA CTA GAA GCC ATA AAA ATC CAA ATC  
 A C L W R Q N N K S S R L E A I K I Q I  
 151/51  
 181/61  
 CTC AAT AAG CTT CCC CTG GAA ACA GCT CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA CTT  
 L S K L R L E T A P N I S K D A I R Q L  
 211/71  
 241/81  
 TTG CCC AAG GCT CCT CCA CTG CGG GAA CTG ATT GAT CAG TAC GAT GTC CAG AGA GAT GAC  
 L P K A P P L R E L I D Q Y D V Q R D D  
 271/91  
 301/101  
 AGC AGC GAC GGC TCC TTG GAA GAC GAT GAC TAC CAC GTT ACG ACG GAA ACG GTC ATT ACC  
 S S D G S L E D D D Y H V T T E T V I T  
 331/111  
 361/121  
 ATG CCC ACG GAG TGT GAT CTT CTA GCA GAA GTG CAA GAA AAA CCC AAA TGT TGC TTC TTT  
 M P T E S D L L A E V Q E K P K C C F F  
 391/131  
 421/141  
 AAA TTT AGC TGT AAG ATA CAA CAC AAT AAA GTA GTA AAG GCC CAA CTG TGG ATA TAT CTG  
 K F S S R I Q H N K V V K A Q L W I Y L  
 451/151  
 481/161  
 AGA CCT GTC AAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCC ATG  
 R P V K T P T T V F V Q I L R L I K P M  
 511/171  
 541/181  
 AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TGT CTG AAA CTT GAC ATG AAC CCA GGC ACT  
 E C G T R Y T G I R S L K L D M N P G T  
 571/191  
 601/201  
 GGT ATT TGG CAG AGC ATT GAT GTG AAG ACA GTG TTG CAA AAC TGG CTC AAA CAA CCT GAA  
 G I W Q S I D V K T V L Q N W L K Q P E  
 631/211  
 661/221  
 TCC AAC TTA GGC ATT GAA ATC AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GCT GTA ACC  
 S N L G I E I K A L D E N G H D L A V T  
 691/231  
 721/241  
 TTC CCA GAA CCA GGA GAA GAA GGA CTG AAT CCT TTT TTA GAA GTC AAG GTA ACA GAC ACA  
 F P E P G E E G L N P F L E V K V T D T  
 751/251  
 781/261  
 CCA AAA AGA TCT AGG AGA GAT TTT GGG CTT GAT TGT GAT GAG CAC TCC ACA GAA TCT CGA  
 P K R S R R D F G L D C D E H S T E S R  
 811/271  
 841/281  
 TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA GCT TTT GGA TGG GAT TGG ATT ATT GCA  
 C C R Y P L T V D F E A F G W D W I I A  
 871/291  
 901/301  
 CCT AAA AGA TAT AAG GCC AAT TAC TGC TCT GGA GAA TGT GAA TTT TTA TTT TTG CAA AAG  
 P K R Y K A N Y C S G E C E F L F L Q K  
 931/311  
 961/321  
 TAT CCT CAT ACC CAT CTT GTG CAC CAA GCA AAC CCC AAA GGT TCA GCC GGC CCT TGC TGT  
 Y P H T H L V H Q A N P K G S A G P C C  
 991/331  
 1021/341  
 ACT CCT ACA AAG ATG TGT CCA ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA  
 T P T K M S P I N M L Y F N G K E Q I I  
 1051/351  
 1081/361  
 TAT GGG AAG ATT CCA GGC ATG GTA GTA GAT CGC TGT GGC TGC TCA TGA  
 Y G K I P G M V V D R C G C S

Ovine GDF-8

FIGURE 14g

M	Q	K	L	Q	M	V	V	I	Y	L	F	M	L	I	A	A	G	P	V	D	L	N	E	G	S	E	R	E	E	N	V	E	K	E	G	L	C	murine		
M	Q	K	L	Q	L	C	V	V	I	Y	L	F	M	L	I	V	A	G	P	V	D	L	N	E	N	S	E	B	O	K	E	N	V	E	K	E	G	L	C	rat
M	Q	K	L	Q	L	C	V	V	I	Y	L	F	M	L	I	V	A	G	P	V	D	L	N	E	N	S	E	Q	K	E	N	V	E	K	E	G	L	C	human	
M	Q	K	L	Q	L	C	V	V	I	Y	L	F	M	L	I	V	A	G	P	V	D	L	N	E	N	S	E	Q	K	E	N	V	E	K	E	G	L	C	baboon	
M	Q	K	L	Q	L	C	V	V	I	Y	L	F	M	L	I	V	A	G	P	V	D	L	N	E	N	S	E	Q	K	E	N	V	E	K	E	G	L	C	porcine	
M	Q	K	L	Q	L	C	V	V	I	Y	L	F	M	L	I	V	A	G	P	V	D	L	N	E	N	S	E	Q	K	E	N	V	E	K	E	G	L	C	ovine	
M	Q	K	L	A	V	Y	V	Y	I	Y	L	F	M	Q	I	A	V	D	P	V	A	L	D	G	S	S	Q	P	T	E	N	A	E	K	D	G	L	C	bovine	
M	Q	K	L	A	V	Y	V	Y	I	Y	L	F	M	Q	I	A	V	D	P	V	A	L	D	G	S	S	Q	P	T	E	N	A	E	K	D	G	L	C	chicken	
M	Q	K	L	A	V	Y	V	Y	I	Y	L	F	M	Q	I	A	V	D	P	V	A	L	D	G	S	S	Q	P	T	E	N	A	E	K	D	G	L	C	turkey	
N	A	C	A	W	R	O	N	T	R	Y	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	K	D	A	I	R	Q	murine
N	A	C	A	W	R	O	N	T	R	Y	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	K	D	A	I	R	Q	rat
N	A	C	T	W	R	O	N	T	K	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	K	D	A	I	R	Q	human
N	A	C	M	W	R	O	N	T	K	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	K	D	A	I	R	Q	baboon
N	A	C	L	W	R	O	N	T	K	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	K	D	A	I	R	Q	porcine
N	A	C	L	W	R	E	N	T	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	K	D	A	I	R	Q	ovine	
N	A	C	T	W	R	O	N	T	K	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	R	D	V	I	K	Q	bovine
N	A	C	T	W	R	O	N	T	K	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	R	D	V	I	K	Q	chicken
N	A	C	T	W	R	O	N	T	K	S	S	R	I	E	A	I	K	I	Q	I	L	S	K	L	R	L	E	T	A	P	N	I	S	R	D	V	I	K	Q	turkey
L	L	P	R	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	murine
L	L	P	R	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	rat
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	human
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	baboon
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	porcine
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	ovine
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	bovine
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	chicken
L	L	P	K	A	P	P	L	R	E	L	I	D	Q	Y	D	V	Q	R	D	D	S	S	D	G	S	L	E	D	D	D	Y	H	A	T	T	E	T	I	I	turkey
T	M	P	T	E	S	D	F	L	M	Q	A	D	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	murine
T	M	P	T	E	S	D	F	L	M	Q	V	D	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	rat
T	M	P	T	E	S	D	F	L	M	Q	V	D	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	human
T	M	P	T	E	S	D	L	L	M	O	V	E	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	baboon
T	M	P	T	E	S	D	L	L	A	E	V	Q	E	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	porcine
T	M	P	T	E	S	D	L	L	A	E	V	Q	E	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	ovine
T	M	P	T	E	S	D	F	L	V	Q	M	E	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	bovine
T	M	P	T	E	S	D	F	L	V	Q	M	E	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	chicken
T	M	P	T	E	S	D	F	L	V	Q	M	E	G	K	P	K	C	C	F	F	K	F	S	S	K	I	Q	Y	N	K	V	V	K	A	Q	L	W	I	Y	turkey
L	R	P	V	K	T	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	murine
L	R	P	V	K	T	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	rat
L	R	P	V	K	T	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	human
L	R	P	V	K	T	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	baboon
L	R	P	V	K	T	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	porcine
L	R	P	V	K	T	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	ovine
L	R	Q	V	Q	K	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	bovine
L	R	Q	V	Q	K	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	chicken
L	R	Q	V	Q	K	P	T	T	V	F	V	Q	I	L	R	L	I	K	P	M	K	D	G	T	R	Y	T	G	I	R	S	L	K	L	D	M	N	P	G	turkey
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K	A	L	D	E	N	G	H	D	L	A	V	murine
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K	A	L	D	E	N	G	H	D	L	A	V	rat
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K	A	L	D	E	N	G	H	D	L	A	V	human
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K	A	L	D	E	N	G	H	D	L	A	V	baboon
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K	A	L	D	E	N	G	H	D	L	A	V	porcine
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K	A	L	D	E	N	G	H	D	L	A	V	ovine
T	G	I	W	Q	S	I	D	V	K	T	V	L	Q	N	W	L	K	Q	P	E	S	N	L	G	I	E	I	K												

FIGURE 15a

32/32

T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		murine
T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		rat
T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		human
T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		baboon
T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		porcine
T	F	P	E	P	G	E	E	G	L	N	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		ovine
T	F	P	E	P	G	E	D	G	L	P	F	L	E	V	K	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		bovine	
T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	R	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		chicken
T	F	P	G	P	G	E	D	G	L	N	P	F	L	E	V	R	V	T	D	T	P	K	R	S	R	R	D	F	G	L	D	C	D	E	H	S	T	E	S		turkey

R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		murine
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		rat
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		human
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		baboon
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		porcine
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		ovine
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		bovine
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		chicken
R	C	C	R	Y	P	L	T	V	D	F	E	A	F	G	W	D	W	I	I	A	P	K	R	Y	K	A	N	Y	C	S	G	E	C	E	F	V	F	L	Q		turkey

K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		murine
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		rat
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		human
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		baboon
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		porcine
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		ovine
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		bovine
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		chicken
K	Y	P	H	T	H	L	V	H	Q	A	N	P	R	G	S	A	G	P	C	C	T	P	T	K	M	S	P	I	N	M	L	Y	F	N	G	K	E	Q	I		turkey

I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																										murine
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									rat	
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									human	
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									baboon	
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									porcine	
I	Y	G	K	I	P	G	M	V	V	D	R	C	G	C	S																									ovine	
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									bovine	
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									chicken	
I	Y	G	K	I	P	A	M	V	V	D	R	C	G	C	S																									turkey	

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

FIGURE 15b

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/02479

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/00, 15/00, 15/09, 15/63  
US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/2; 435/172.3, 69.1, 320.1, 325; 530/350, 387.1; 514/2, 44; 424/9.21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MCDOWELL et al. Effects of Exogenous Growth Hormone on Milk Production and Nutrient Uptake by Muscle and Mammary Tissues of Dairy Cows in Mid-Lactation. Australian Journal of Biological Sciences, Vol. 40, No. 3, pages 295-306, see Abstract.	13
Y	EVOCK et al. Pituitary Porcine Growth Hormone (pGH) and a Recombinant pGH Analog Stimulate Pig Growth Performance in a Similar Manner. Journal of Animal Science, Vol. 66, No. 8, pages 1928-1941, see Abstract.	14

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 MARCH 1998

Date of mailing of the international search report

15 JUN 1998

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/02479

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FLAKOLL et al. Influence of Alpha-Ketoisocaproate on Lamb Growth, Feed, Conversion, and Carcass Composition. Journal of Animal Science, Vol. 69, No. 4, pages 1461-1467, see Abstract.	15
Y	DELI et al. Biochemical Study of Muscle Samples from Chicken Embryos Affected by Wofatox 50 EC. Archives of Toxicology, Vol. 8, pages 277-279, see Abstract.	16
Y	FAULKNER et al. Effect of Testosterone Propionate on Performance and Carcass Characteristics of Heifers and Cows. Journal of Animal Science, Vol. 67, No. 8, pages 1907-1915, see Abstract.	12
Y	ZHU et al. Survey of Major Histocompatibility Complex Class II Haplotypes in Four Turkey Lines Using Restriction Fragment Length Polymorphism Analysis with Nonradioactive DNA Detection. Poultry Science, Vol. 74, No. 7, pages 1067-1073, see Abstract.	11

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/02479

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

800/2; 435/172.3, 69.1, 320.1, 325; 530/350, 387.1; 514/2, 44; 424/9:21

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS, CONFSCI

search terms: growth differentiation factor-8, GDF-8, transgene, increased muscle, reduced cholesterol, mouse, pigs or porcine, cows or bovine, sheep or ovine, piscine, chicken or turkey or avian